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# EVOLUTION UNDER DOMESTICATION

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Evolution is usually regarded as a process which moves with a majestic and unhurried pace through geological periods and eras and whose progress is measured in millions of years. Evolution is ordinarily thought of as so slow that even the second hand on the evolutionary clock does not travel perceptibly in the span of a human lifetime, or, in many cases, during the period of recorded human history. There is, however, one kind of evolution which does not conform to this general rule and which often proceeds with extraordinary rapidity. This is the evolution of animals and plants under domestication.

A century ago Charles Darwin, the father of modern evolutionary theory, was keenly interested in the evolution of animals and plants under domestication and he gained many of his ideas on the factors involved in evolution —induced variation, inbreeding, hybridization and selection—from his studies on it. Darwin realized that variation in cultivated plants and domestic animals if not identical with that in nature is at least its counterpart. He seemed to feel, if I interpret him correctly, that a study of evolution in domestic animals and plants offers a kind of magnified view of evolution in general, thus revealing for processes too slow to be readily examined in one observer's lifetime approximately what the microscope does for objects too small to be studied in detail with the naked eye, or what the telescope can do for heavenly bodies too distant to be clearly seen. There is at least no doubt that Darwin was tremendously interested in animals and plants under domestication and that he made an intensive study of the subject. He could not have lived at a better time for making such a study. Many of the modern breeds of livestock, cattle, sheep, chickens, dogs and pigeons were coming into existence under the hands of skilled breeders who employed inbreeding, hybridization and selection as tools of their profession. Horticulturists were bringing together species of plants from all parts of the world and by hybridization and selection were breeding spectacular new forms. Darwin followed these developments avidly. Upon his return to England after the voyage of the Beagle where the facts of variation in nature were indelibly impressed upon him and

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where he had already begun to give serious thought to the subject of modification of species, Darwin turned his mind upon all the kinds of evidence which might have a bearing upon the problem and among these he gave a position of pre-eminence to the data from domestic animals and plants. A brief quotation from one of his biographers (Trattner) describes one of his principal activities of this period.

> So Darwin went about the countryside talking to professional breeders of domestic animals and plants, horticulturists, cattlemen, farmers, cat and dog fanciers. He put questions to each of them: what qualities did they breed for, how successful were they in perpetuating them, what were the especial virtues of hybrids, how did domesticated species differ from wild? He learned that it was comparatively easy for breeders to modify a domestic species within a few generations (British cattlemen for example had improved the quality of cattle and sheep to a marked degree within a short time and race-horse trainers had so improved the original stock of Arabian horses that pure Arabians had to be given handicaps in the races). Wherever he inquired he saw instances of man's ability to improve nature by artificial selection. Domestic breeders had produced better grades of cattle and sheep. So Darwin jotted down in his notebook that "the power of this principle of selection is not hypothetical."

When Darwin finally put to paper the ideas which he had been working over in his mind for so many years and the vast amount of evidence which he had accumulated, his data on domestic animals and plants and the conclusions which he drew from them occupied a prominent part in his arguments. The first chapter in his epoch-making book, "The Origin of Species," is devoted to this subject and he subsequently wrote a two-volume treatise on the variation of animals and plants under domestication. Darwin believed that the key to the problems of modification and adaptation was to be found in the study of variation of organisms under domestication. A passage in the first chapter of his "Origin of Species" makes this abundantly clear.

> At the commencement of my observations it seemed to me probable that a careful study of domesticated animals and of cultivated plants would offer the best chance of making out this obscure problem. Nor have I been disappointed; in this and in all other perplexing cases I have invariably found that our knowledge, imperfect though it be, of variation under domestication, afforded the best and safest clue. I may venture to express my conviction of the high value of such studies, although they have been very commonly neglected by naturalists.

Domestic animals and cultivated plants are still important as materials for studies of evolution, but their role is not what it was in Darwin's time. Genetics passed a major milestone when Wright and other population geneticists, especially Dobzhansky, Fisher and Haldane, showed by mathematical analysis, far more rigorous than is possible with verbal reasoning, that the principal facts of evolution are entirely explicable in terms of Mendelian heredity. Studies of evolution under domestication are not likely, therefore, to reveal, as they did in Darwin's hands, any new principles. They can still serve a useful purpose, however, in testing the conclusions reached by mathematical analysis and in determining how the genetic agents of evolution act and interact in an environmental framework quite different from that occurring in nature. It is the purpose of this paper to examine these several possibilities.

Evolution is defined by population geneticists as changes in gene frequencies, and four genetic agents—mutation, random genetic drift, hybridization and selection—are recognized as working these changes. These are not the only factors involved in evolution but all other factors such as isolating mechanisms and geological, climatic and other changes in the environment work through these four genetic forces. Under domestication a new factor, man, has been superimposed upon the genetic agents and has accentuated and exaggerated the action of each one to a remarkable degree.

The intervention of man in the process of evolution on a grand scale is a comparatively recent event in the history of the world which occurred when agriculture was invented and a nomadic, hunting and food-gathering culture was transformed into a food-growing one. How long ago this occurred is still a subject for debate. So far as tangible archaeological evidence goes, however, the event must have been comparatively recent. The oldest remains of cultivated plants from the Near East are dated by archaeologists using conventional methods at 5000 to 6000 B. C. Determinations of residual radioactive Carbon 14 by Libby and his colleagues have now given us a valuable new tool for estimating the age of archaeological remains. Such estimates place the oldest remains of cultivated plants so far studied by this method, from the Old World—specimens of wheat and barley from the Near East—at 6095 years ± 250. The oldest remains of cultivated plants in the New World, corn cobs from Bat Cave in New Mexico, are estimated on the basis of determinations, not of the cobs themselves, but of wood charcoal associated with them at 5931 ± 310 years. Older remains than these will undoubtedly be found as the result of future archaeological excavations and perhaps agriculture will be shown to have had its beginnings as much as 10,000 years ago. Even this period of time is so brief in terms of evolutionary progress that the changes effected in domesticated animals and plants seem remarkable indeed and all the more remarkable when we consider that many of them are the products not of 10,000 years of gradual evolution but of man's conscious efforts during the past 200 years. How is it that the corn plant, for example, could have been so drastically altered in a few thousand years while certain genera of brachypods are said by Simpson not to have changed appreciably in 400 million years? The answer lies in the activities of man. In the practice of agriculture man has transformed the surface of the earth and made cataclysmic changes in its vegetation. From the world's quarter of a million species of plants he has chosen a few hundred, most of which were probably not faring too well in nature and has created for these chosen species a new man-made environment. He has removed the natural vegetation, altered the physical, chemical and biological properties of the soil, protected his exotic crop plants from pests and diseases, and through the subsequent invention of irrigation has literally changed the climate in which they grow. In this new environment the struggle for existence with other species has been almost eliminated. Competition under domestication is largely intraspecific and competition between species is reduced to a minimum. The chief criterion

for an individual's survival is whether it meets the requirements of man. If it does it can reproduce prodigiously; if it does not it is destined to extinction. And as man has moved over the face of the earth carrying his cultivated plants and domestic animals with him wherever he went, exchanging his own varieties with those of other peoples, he has opened up thousands of new niches in which his favored species not only could but were compelled to evolve along new paths. The magnitude of these new evolutionary opportunities is difficult to visualize. The wheat plant, for example, which once occupied a restricted range somewhere in the Near East or Central Asia in competition with numerous other grasses, now grows on 418 million acres in all parts of the world virtually free of competition with other species. The maize plant, which perhaps was once on the verge of extinction in an isolated locality in eastern South America or in Central America, now occupies 221 million acres in all parts of the world where agriculture is practiced. There is no counterpart of this situation in nature. Let us see how the four principal genetic agents, mutation, genetic drift, hybridization and selection, have worked to adapt cultivated plants and domestic animals to the new niches which man has opened up to them. And if in discussing this subject I should refer to plants more frequently than to animals it is only because my own experience has been botanical rather than zoological and if among plants I should use maize more frequently as an example than any other, it is not only because maize furnishes excellent illustrative material but also because having now worked with it for thirty years, I know it somewhat better than I know any other plant.

Under domestication, as in nature, the building blocks of evolution are mutations. There is no other primary source of variation. There is no evidence to support the common belief that domestication itself creates new hereditary variations. One of Darwin's greatest errors was in concluding that it does. He was convinced that organisms vary more widely under domestication than in nature and he regarded an excess of food in both animals and plants as the most powerful single agent causing increased variability. He believed that the variations which appear under domestication are inherited, at least in part, and it was this belief that led him to develop his untenable theory of pangenesis to account for the inheritance of acquired characters. Darwin was right in concluding that domesticated species as a rule vary more widely than species in nature, but this fact can now be satisfactorily explained in modern genetic terms.

It is quite probable, though difficult to prove by direct evidence, that domesticated species have been derived from wild species which were already, while still in nature, more mutable than species in general. This conclusion is reached by inference. If in species which are well adapted to their environment the mutation rate in nature is low and is kept so by the pressure of natural selection, then in species which are not well adapted to their environment it is quite likely to be above the average. The wild species with which man began domestication were often species not especially well fitted for survival in nature and indeed some were undoubtedly

moving toward extinction when man appeared on the scene. There is not a single species of animal or plant, so far as I know, which is widely distributed both in nature and under domestication and there is a rather strong negative correlation between the distribution of species under domestication and in nature. Such plants as maize and the bread wheats which have a world-wide distribution as cultigens are today unknown in the wild. Their original range must have been very limited indeed.

If highly successful domesticated species have descended from species which were poorly adapted in the wild as I have suggested, then it is likely that these species had become mutable because the pressure of natural selection on the rate of mutation was no longer strongly operative in them. If so, domestication exploited the mutation potential far beyond that possible in nature. Millions of plants now grew in cultivated fields where only hundreds had grown in nature. The opportunities for mutations were increased in direct proportion to the increase in population size and the mutations which now occurred came under man's purview. If they were useful to him or of interest to him through their novelty or bizarreness they were preserved.

Two other evolutionary agents, genetic drift and hybridization, may well have interacted to increase the mutation rate under domestication by creating new populations in which mutation suppressors were either reduced in number or eliminated. Natural selection operating in the environment of domestication shaping the species to countless new niches may well have favored populations with a low frequency of mutation-suppressing genes and hence a high mutation rate.

Direct evidence that domesticated species are derived from mutable wild species or even that the mutation rate is high in domesticated species is almost completely lacking. Stadler has given us some interesting data on spontaneous mutation rates for specific loci in maize but we have nothing in nature with which to compare them. The genes studied by Stadler mutated at rates varying from 0 to 492 per million gametes. (Singleton has recently found much higher rates for some of the same genes.) I have found that the spontaneous mutation rate at the tunicate locus in maize is quite high. The higher spontaneous rates in maize are of the same order as x-ray induced mutation rates at the white locus in Drosophila (about 510 per million) and these in turn are about 60 to 70 times the natural rates at the same locus. It is possible to conclude from these comparisons that maize, if not a mutable species, at least contains some highly mutable loci.

There is other indirect evidence that domesticated species are sometimes highly mutable. Pure lines of self-fertilized plants such as wheat, oats and barley do not long remain pure. Although appearing to retain their uniformity in their principal morphological characteristics, they often prove to be heterozygous in resistance and susceptibility to new races of rust and other diseases when experimentally inoculated. Jones at the Connecticut Station found that sib lines of maize separated after seventeen generations of inbreeding were significantly different, presumably because of mutations

rather than because of residual heterozygosity. Some of these old lines of maize apparently became variable through mutations more rapidly than they were becoming uniform through inbreeding. East observed some years ago that a diploid Nicotiana derived from a spontaneous haploid and therefore completely homozygous for all of its genes was initially quite uniform but began almost at once to vary, apparently as the result of numerous mutations with small effects.

Are the mutations which occur under domestication the same kind that occur in nature? Presumably they are but again there is little direct evidence and no proof. There has, however, undoubtedly been a difference in the fate of mutations in the two environments. Mutations with manifold effects, sometimes involving monstrous changes—mutations approaching the "hopeful monster" of Goldschmidt—which would have little or no chance of survival in nature, may be preserved under domestication because they are more useful to man or because they are valued for their novelty, bizarreness or imagined magical properties.

The preservation of monstrous forms is well illustrated by the cultivated Cruciferae related to cabbage. The wild cabbage plant which still grows in the coastal regions of Europe and Northern Africa has given rise to cabbage with its monstrous terminal bud, to cauliflower and broccoli with their monstrous modified edible inflorescences, Brussels sprouts with a monstrous constellation of lateral buds and kohl rabi with a monstrous swollen stem. Only a relatively small number of genes are involved in these domesticated botanical monstrosities. Heading of cabbage, for example, is a matter of two or perhaps three pairs of genes. The swollen stem of kohl rabi involves two major factors and a pair of modifying factors.

In maize there have been a number of mutations which have come close to meeting the specifications of Goldschmidt's "hopeful monster"—mutations which affect the entire organism so profoundly that something resembling a new species is created literally overnight. Perhaps the most striking of these is Singleton's "corn grass," a dominant mutation which transforms a tall, single-stalked, broad-leaved plant into a low-growing, freely-tillering, narrow-leaved bunch grass with numerous floral modifications, a form which if found growing as a colony in the wild might well have been described as a new species if not a new genus. Singleton suggests that maize as we know it may have originated from a wild grass of this kind by a single macromutation from the dominant to the recessive condition. I doubt this, since corn grass lacks one very important characteristic which wild maize, like other cereals, must certainly have had, protection of its kernels by glumes. I think it far more likely that cultivated maize has originated by a single macromutation from pod corn which does possess this essential ancestral characteristic.

Pod corn, which is still found as an admixture in the indigenous maize varieties of the Indians of both North and South America, is the product of a single dominant gene with numerous effects. It not only causes the kernels to be enclosed in glumes but it tends to suppress the lateral in-

florescence, the ear, thus diverting much of the plant's energy into a massive terminal inflorescence bearing both male and female flowers. If in addition to this terminal inflorescence there is also an ear it often has an elongated rachis and shank, is sometimes branched and is frequently only partially enclosed in unmodified leaf sheaths bearing normal blades. The several effects which the pod-corn gene produces allow us to visualize an ancestral form which meets all the requirements of a wild plant and possesses all of the botanical characteristics which would be expected to occur in a species of the Maydeae, the tribe of grasses to which corn belongs. The ancestral form thus reconstructed has glume-protected seeds borne on the central spike as well as basally upon the branches of a terminal inflorescence. It resembles rather closely a wild relative of corn, Tripsacum. The mutation from pod corn to the non-podded form would not only have reduced or eliminated the glumes but would also have promoted the development of lateral shoots. These, bearing terminal, largely pistillate, often branched and partly naked inflorescences would have been useful to man but highly vulnerable to insects and birds. Natural selection acting upon this new character would rapidly have modified it into the ear as we know it, the most characteristic organ of the modern maize plant. The branches were lost, leaving only the central spike; the stalk contracted to become a short shank; the leaf sheaths became broader and thinner and lost the larger portion of their blades. An inflorescence in which each kernel was separately enclosed in glumes thus became in a few steps one in which all the kernels were enclosed *en masse* in husks. All of these supposed steps can still be seen in indigenous Indian varieties of maize especially those of South America and occasionally we may see the complete transition occurring as a single step in one plant.

The tunicate gene whose mutation is assumed to have produced part of these drastic changes and to have set in motion others is a mutable gene. I have discovered four separate mutations at the tunicate locus in my experimental cultures which have usually not included more than a few hundred tunicate plants each season. In one controlled experiment to test the mutation rate at the tunicate locus it was found to be 471 per million gametes.

A macromutation such as that from pod corn to naked corn has an evolutionary significance beyond that of producing a change in the morphological nature of the organism. If it is preserved by man it immediately places the plant in a completely new relationship to its environment, a relationship which demands numerous additional changes and which allows still others. Selection for modifying factors would be expected to proceed at a rapid rate adapting and perfecting the "hopeful monster" to the new niche, which in a sense, it has created for itself.

In spite of the fact that corn may have originated from a macromutation in the Goldschmidtian sense, I see in this circumstance little evidence that macromutations or "hopeful monsters" are a factor in evolution in nature. Indeed if pod corn or Singleton's corn grass suggest anything, they indicate that macromutations are important *only* under domestication where

new forms, however bizarre and monstrous, can be, and are, perpetuated and preserved under man's protection.

Random genetic drift, the term applied to changes in gene frequencies resulting from random sampling of gene populations, was not known to Darwin as an evolutionary factor. Population geneticists today are not in complete agreement on its importance in nature but there can be little doubt of its effectiveness under domestication. This is probably to be expected. Any gene population which has been broken up so rapidly and repeatedly as the result of man's movements, as have those of our domesticated species, are almost certain to have felt the effects of random sampling. There is abundant evidence that they have. Vavilov, Russia's and this century's greatest student of diversity in cultivated plants, recognized this phenomenon, although he did not call it genetic drift and he employed it systematically as one means of determining centers of origin. He concluded from his world-wide studies that recessive genes with a low frequency at the center of a plant's origin, may suddenly achieve a high frequency at the periphery of its spread, simply as a result of random sampling, or as Vavilov called it, the "emancipation" of recessives. Russian botanists have continued to use this criterion in studying the distribution of cultivated plants and when there are no disturbing factors it has proved to be a useful one.

The effect of genetic drift is immediately apparent to plant breeders working in such a country as Mexico where agriculture is practiced in scores of mountain valleys more or less isolated from each other. The natives of one valley may have a productive race of maize capable of making efficient use of the environmental potentials. The natives of an adjoining valley with a similar environment may have a race which for no apparent reason except genetic drift yields substantially less. The plant breeder attempting to improve the agriculture of the country as a whole will, as a first step, compare the different races under uniform conditions and will then distribute the better races throughout the regions to which they are adapted. Thus, the most fruitful first step which the plant breeder can take in improving the maize of a country such as Mexico is to neutralize the effects of past genetic drift.

The phenomenon of genetic drift in maize is also illustrated in the distribution of certain genes which, so far as we can determine, have no adaptive value. The *Pr* gene on the fifth chromosome in maize is responsible for purple aleurone color in the kernel but only when three other genes, *A*, *C* and *R*, are present. Since either *C* and *R* are usually lacking in the majority of cultivated races of maize and since *Pr*, in the absence of its complementary genes has no opportunity to act, there is no apparent reason why there should be differences in the frequency of this gene in different parts of America. There are, however, actually very great differences in its frequency which varies from 30 per cent in Panama to 98 per cent in the Corn-Belt of the United States.

A similar situation exists with respect to the *I* gene on the ninth chromosome which completely inhibits aleurone color when it would ordinarily be

produced by the action of *A*, *C* and *R* alone or in combination with *Pr*. There may have been a time in the evolution of maize when this gene served a useful purpose, but now when in the majority of races there is no aleurone color to inhibit, its presence can be detected only by making crosses with appropriate tester stocks. As in the case of the *Pr* gene there is no apparent reason why there should be differences in its frequency in different parts of the world since it, like the blood groups of man, has no apparent adaptive value, yet there are marked differences; its frequency in different regions varies from zero per cent in Corn-Belt dent corn to 77 per cent in Honduras.

When we consider all of the nonadaptive genes whose frequencies we have studied in this way, we find that the maize of the United States, the world's most important center of maize production, is not at all typical of maize in general and that it represents almost a unique sample of the diversity which exists in this species. Its uniqueness is due, at least in large part, to the phenomenon of genetic drift.

Hybridization, whose importance Darwin clearly recognized, is a much more powerful force in evolution under domestication than it is in nature. Natural species are, by definition, groups of individuals which do not readily exchange genes with one another and there are in nature many mechanisms to promote and preserve the isolation which has grown up between species, genera and higher categories of classification. Under domestication, where evolution occurs within the species, there are few or no isolating mechanisms except those imposed by geography or consciously or unconsciously by man. All races of maize or breeds of dogs are completely interfertile and this is true also of the majority of domesticated species.

Interspecific hybridization has, it is true, played an important role in the origin of cultivated plants but primarily in suddenly creating new species which have been more amenable to domestication or in other respects more useful to man than their parents. The cultivated wheats, cottons, tobaccos and many other cultivated species are allopolyploid forms resulting from hybridization either in nature or under domestication. The cultivated bread wheats may be an example of the latter. The principal species of wheat, *Triticum aestivum*, is unknown in the wild. Its twenty-one chromosomes comprise three distinct genoms of seven chromosomes each derived from three wild grasses once geographically separated but brought into proximity through man's peregrinations. Thus, it is possible that all the billions of wheat plants now grown on millions of acres of land throughout the world trace their origin to a single spontaneous allopolyploid hybrid plant which appeared in the field of a primitive farmer less than 10,000 years ago.

Hybridization has been important in setting species on the road to domestication and it has been even more important in shaping their evolutionary development under domestication. Both inter- and intraspecific hybridization have been involved and it would be difficult to say which has been the more important. Many of our horticultural plants, especially those propagated vegetatively, are the products of hybridization between species.

Many of our field crops propagated by seed show the effects of hybridization within the species.

Hybridization produces two principal effects. Through Mendelian recombination it creates new diversity upon which both natural and artificial selection may act. And it also produces hybrid vigor or heterosis. The hybrid races of maize in Mexico which came into existence naturally are gradually replacing their older component races because they are more vigorous and productive. Hybrid vigor persists beyond the first generation partly, perhaps, because so many genes are involved in the phenomenon that the modal genotypes of the hybrid population are essentially as heterozygous as the first generation hybrid. And perhaps in addition natural selection operates, as Dobzhansky has shown that it does in Drosophila, to maintain heterozygosity. There is at least no doubt that modern races of maize are highly heterozygous or that repeated hybridization has played an important role in the evolutionary history of this species.

During the first few thousand years of domestication hybridization was largely an accidental factor in evolution in which man played no part except as he, by carrying his plants and animals to all parts of the world, unconsciously destroyed the geographical barriers which had previously separated different gene populations. With the advent of systematic plant breeding geographical barriers to hybridization have been consciously destroyed. The plant breeder brings together genes from the world over in producing new varieties. The principal wheat variety of Minnesota, Newthatch, is an example. It is the product of combining the most desirable agronomic characters of varieties of wheat from all parts of the world. Newthatch has in its ancestry races of wheat from India, Galicia, the Crimea and other parts of the Near East.

The conscious exploitation by man of the factor of hybridization and the heterosis which so often accompanies it has reached its culmination in the development of the hybrid corn which now occupies three-fourths of all the corn acreage in the United States and is spreading to Italy, Mexico and other countries where corn is an important crop. In producing hybrid corn man has artificially created on a vast scale a kind of adaptive polymorphism similar to that found in Drosophila in nature in which certain genotypes are preserved not for their intrinsic worth but because they combine effectively with other genotypes similarly preserved to maintain a high degree of heterozygosity in the population as a whole.

Finally we come to selection as a factor in evolution under domestication. Darwin has made it quite clear that his theory of natural selection as the principal modifying force in evolution grew out of his observations on the power of human selection upon domestic animals and cultivated plants. Speaking of the species of the Galapagos and their differences with those of the American mainland he stated,

> But it long remained to me an inexplicable problem how the necessary degree of modification could have been effected, and it would have thus remained for ever, had I not studied domestic productions, and thus acquired a just idea of the power

of Selection. As soon as I had fully realized this idea, I saw, on reading Malthus on Population, that Natural Selection was the inevitable result of the rapid increase of all organic beings; for I was prepared to appreciate the struggle for existence by having long studied the habits of animals.

Nor was there any doubt in Darwin's mind that human selection and natural selection were essentially similar evolutionary processes. He stated:

As man can produce and certainly has produced a great result by his methodical and unconscious means of selection, what may not nature effect? Man can act only on external and visible characters: nature cares nothing for appearances, except in so far as they may be useful to any being. She can act on every internal organ, on every shade of constitutional difference, on the whole machinery of life. Man selects only for his own good; Nature only for that of the being which she tends. Every selected character is fully exercised by her; and the being is placed under well-suited conditions of life. Man keeps the natives of many climates in the same country; he seldom exercises each selected character in some peculiar and fitting manner; he feeds a long and a short-beaked pigeon on the same food; he does not exercise a long-backed or long-legged quadruped in any peculiar manner; he exposes sheep with long and short wool to the same climate. He does not allow the most vigorous males to struggle for the females. He does not rigidly destroy all inferior animals, but protects during each varying season, as far as lies in his power, all his productions. He often begins his selection by some half-monstrous form; or at least by some modification prominent enough to catch his eye, or to be plainly useful to him. Under nature, the slightest difference of structure or constitution may well turn the nicely-balanced scale in the struggle for life, and so be preserved. How fleeting are the wishes and efforts of man! how short his time! and consequently how poor will his products be, compared with those accumulated by nature during whole geological periods. Can we wonder, then, that nature's productions should be far "truer" in character than man's productions; that they should be infinitely better adapted to the most complex conditions of life, and should plainly bear the stamp of far higher workmanship?

Today we still recognize selection as one of the powerful forces, in some circumstances the most powerful force, in evolution in nature. Selection is equally important under domestication and in some instances much more so. Nor is there any more reason now than there was in Darwin's time to believe that any essential difference exists between natural and artificial selection except that the one occurs naturally while the other is practiced consciously or unconsciously by man.

Until recent centuries selection under domestication must have been primarily natural selection acting in a man-made environment. The force remained the same, only the direction had changed. In animals, docility, a useless character in nature, if not a highly deleterious one, suddenly found itself under domestication possessing substantial survival value. In wild grasses individuals which had through mutation lost the mechanism for dispersing their seeds, were at a great disadvantage and did not long persist. In cultivated cereals derived from these same wild grasses individuals which retained their seed until the primitive farmer harvested the crop had a much better chance of perpetuating themselves and even in the absence of human selection would soon have replaced the dehiscent forms. Natural selection would have, and indeed still does, act in the same way upon many other characteristics and it often acts with remarkable rapidity.

The effectiveness of natural selection in cultivated plants is well illustrated by the rapidity with which open-pollinated varieties of maize become adapted to new environments. Kiesselbach and Keim showed thirty years ago that in Nebraska, which had then been settled only about a century, the maize varieties of the state had become so different in their adaptation that growing them at new stations only a few hundred miles east or west usually resulted in substantial reductions in yield. Natural selection is also illustrated by experiments such as those of Harlan and Martini on growing mixtures of several varieties of cereals in different localities. In each locality one component in a few generations dominates the mixture.

Natural selection is effective in a man-made environment, but artificial selection is even more so. Some students of domestication believe that man has practiced artificial selection since its beginnings. There is little evidence to support this view. There are, of course, Biblical references to the selection of domestic animals and the ancient writers on agriculture of Greece and Rome discussed the practice of selection in both animals and plants. But these are the attributes of civilization already highly advanced and not of primitive cultures. Primitive man seems often to accept, with little or no effort on his part to change, the populations which natural selection and random genetic sampling have presented to him. If he recognizes different degrees of excellence he is all too likely to consume first that which he considers best and to use for seed whatever remains. That cultivated plants have improved in spite of these disgenic practices testifies to the powerful force of natural selection interacting with the other genetic agents of evolution.

The apparent absence of artificial selection in the early stages of domestication is well illustrated by 3000 years of evolution of maize as it is revealed by archaeological remains discovered in Bat Cave in New Mexico. Although these remains show an increase in the average size of the ear and kernel during this period, the small primitive corn which was present at the beginning did not become extinct even at the end as it surely must have done had rigorous selection in favor of large ears been constantly practiced.

But if conscious selection was a minor factor in the early centuries of domestication it has become a major one in modern times. Indeed there has probably been more modification of our cultivated plants and domestic animals in the past two centuries than in all the previous millennia of domestication. Selection in the hands of man can be an almost unbelievably powerful force. This is illustrated both by experience and experiment. The stock beet which was grown in Germany for cattle feed had a sugar content of 7.5 per cent when Koppy in 1810 began his experiments on using it as a source of sugar. Koppy had by 1820 raised this to 10—11 per cent. The sugar percentage was raised further to 16—17 per cent by Vilmotin by 1861. The wild rubber tree of Brazil, *Hevea brasiliensis*, growing in its native jungle yielded only a few pounds of rubber per acre. Selected clones in plantations produce about 400 pounds per acre and new clones in experi-

mental cultures have produced at the rate of 2000 pounds per acre. Dairy cows descended from animals which secreted only enough milk to nurse one calf are producing twenty to thirty times their own weight in milk each year. Chickens whose ancestors a few thousand years back laid one or two clutches of twelve to fifteen eggs each have been bred to lay more than 300 eggs a year. In the classical experiments on selection for chemical composition in maize at Illinois University protein content was raised from 10.9 to 14.5 per cent in nine generations and in other families lowered to 7.7 per cent. Oil content was raised by selection from 4.7 to 8.5 per cent and lowered to 2.0 per cent.

These four evolutionary forces, mutation, genetic drift, hybridization and selection, interacting to a degree seldom encountered in nature and accelerated and intensified by the activities of man have produced evolutionary changes so profound and in so short a time that a paleontologist seeing the species only at the beginning and the end might well suspect that evolution under domestication is cataclysmic and the product of violent saltation. When we observe the intervening steps, as in some instances we can, we see that this is not true and we see also that evolution under domestication is the exact counterpart of evolution in nature, the product of what Wright calls the "interplay of directed and random processes." Man has played an important part in it, but perhaps more important still is its impact upon him. It can be said with some degree of truth that man's rise from a state of savagery to one of civilization began when he accidentally set in motion and himself became involved in the genetic forces of evolution acting upon animals and plants under domestication.

I had hoped to conclude this paper with a discussion of the most interesting of all domestic species, *Homo sapiens*, and to consider what implications the facts of evolution under domestication hold for him, but space permits no more than a brief glance at this intriguing subject. Man became a domestic animal when he invented agriculture and created an artificial environment filled with countless new niches not only for his animals and plants but for himself. The parallels between man and his domesticated species are obvious and the implications are apparent. But when I contemplate the potential power which man holds in his hands to shape the human species as he has shaped others and compare with it his present state of wisdom, the implications are distinctly frightening. A more hopeful aspect lies in the fact that for 400 to 500 generations the forces of evolution have been creating diversity in the human species and if this natural trend is permitted to continue its course even greater diversity is in store for mankind. And in this changing world diversity, whether biological or political, is perhaps the surest safeguard against extinction.

# THE PATTERN OF DIFFERENTIATION IN AMOEBOID SLIME MOLDS*

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Princeton University

In the latter part of the nineteenth century Hans Driesch discovered the remarkable fact that if parts of a sea urchin embryo were isolated, then each separate part would produce a perfect, complete, diminutive individual. He called such a system harmonious and equipotential; harmonious because each part somehow was integrated to become a whole, and equipotential because all the parts of the original embryo proved upon isolation to be capable of giving rise to all the differentiated structures of an individual larva. It is an unfortunate historical fact that Driesch is remembered not so much for his deep insight into the problem of regulatory development, but for his philosophical conclusions. He found this kind of development so astounding and so remote from human experience that for him the only possible explanation was a vitalistic one, involving an entelechy. The very fact that the embryo can be subdivided and each part be a harmonious equipotential system proved in his eyes that no mechanistic interpretation could account for the facts, for what machine, he said, will reorganize to make up for the loss of essential parts? But nowadays our reverence for machines has increased many fold and the mathematicians talk of electronic calculating machines that play good chess and can perform various types of involved thinking—a far cry from the mechanical toys that so impressed Descartes. If Driesch had seen these remarkable achievements of today he might have been restrained from his entelechies, although he still might have asked whether cutting a huge calculator in two will give two perfect dwarf calculators. No doubt the mathematicians have an answer, but it is not my purpose here to discuss regulation in electronic calculators.

Instead, I wish to discuss an organism, *Dictyostelium discoideum,* which more than any other living form exposes the problem of regulation. The basis for this rather sweeping statement lies in the very nature of its life cycle, for regulation takes place in a mass of cells which has arisen by aggregation, and not in a cell mass that arises directly by growth and cell division from an egg. As will be shown the cells of the mass may arise from one or many clones and the only factor which determines their ultimate differentiation is their position within the mass, following the well-known principle established by Driesch.

* Presented at the Symposium of the American Society of Naturalists on "Patterns of Cellular Organization," Minneapolis, Minn., September 12, 1951.

[1] The new experimental work presented in this paper was carried out with the help of a grant from the American Cancer Society and was also supported in part by funds of the Eugene Higgins Trust allocated to Princeton University. I also would like to record my indebtedness to Mrs. Evelyn Frascella for her able assistance in this work.

The spores of *Dictyostelium discoideum* consist of small, smooth capsules each of which contains a single uninucleate amoeba and upon germination the capsule splits and the amoeba emerges. (See Raper, 1935, 1940a; Bonner, 1944, for details of the life cycle.) This amoeba will feed by engulfing bacteria and as it increases in size it will divide by binary fission so that in the course of time there will be many separate independent amoebae in this vegetative stage. When the food supply is depleted, and even more important when a critical concentration of amoebae is reached, they will begin to aggregate, begin to stream together to central collection points, and apparently the amoebae are guided by a gradient of a chemical substance called acrasin to which they respond chemotactically (Bonner, 1947). The cell aggregate assumes a sausage shape usually 1 or 2 mm. long, and proceeds to migrate over the substratum for variable periods of time depending on the external conditions.[2] At the end of migration the sausage rights itself and pushes up into the air (culmination stage) to form a fruiting body consisting of two cell types: the apical ball of capsulated spore cells and the thin tapering stalk made up of large vacuolate cells enclosed in a smooth cylinder of cellulose.[3] It will now be helpful to trace the steps involved in this final differentiation in more detail.

By the use of some ingenious coloration experiments, Raper (1940b) was able to determine the normal fate of various parts of the migrating cell mass. He found that the anterior portion gives rise to the stalk and the posterior region gives rise to the spore mass. Later it was found possible (Bonner, 1944), by the use of stained paraffin sections, to see in advanced migrating cell masses differences in the appearance of presumptive spore and presumptive stalk cells; the former were small and stained densely with haemotoxylin, and the latter were larger and paler after staining. The most remarkable aspect of these sections was that the division line between these early spore and stalk cells was extremely sharp. (See Bonner, 1944, fig. 1.) Following the subsequent stages in the paraffin sections one could clearly see that these prestalk cells first became true vacuolated stalk cells in the tip and that this tip was pushed like a wedge downward through the center of the presumptive spore mass. I have seen this process clearly in a recent experiment in which the anterior cells were stained with the pigment of *Serratia marcescens* (using the technique of Raper, 1940b), and the pushing down of the wedge may be followed (fig. 1). The remaining presumptive stalk cells push upward to the tip of the stalk and as they arrive in this apical position they become trapped and soon enlarge and become vacuolate. So by a combination of the progressive piling of cells on the top of the stalk and their vacuolization the stalk rises into the air. Attached to the apical end of the stalk remains the spore mass, the majority of the

[2] Studies on the factors affecting the duration of migration are now being carried out in collaboration with Mrs. M. K. Slifkin. Methods have been devised to keep migration going for a period as long as 20 days in one instance.

[3] There is also a basal disc peculiar to *D. discoideum* containing vacuolate cells which surround the base of the stalk.

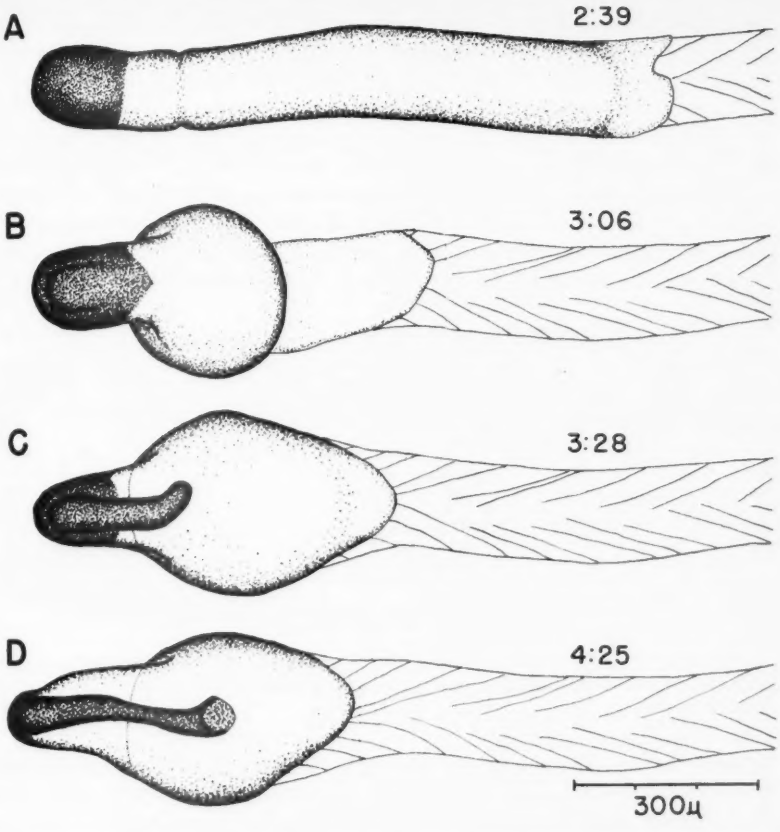


FIGURE 1. Camera lucida drawings (surface views) showing how the stalk is first formed at the tip and is pushed downward through the pre-spore cells to the substratum. The dark tip was obtained by grafting the tip of a colored migrating cell mass onto a decapitated colorless one.

pre-spore cells having differentiated into true spores rather early in the rise upward.[4]

During this past year a new method of observing the division line between presumptive stalk and presumptive spore cells during migration has been found. The advantage of this particular method is that early differentiation may be observed in the living condition. We simply suspend in water large quantities of vegetative amoebae and stain them with a vital dye; neutral red, Nile blue sulphate, and Bismarck brown being the most useful ones.[5] Since all the amoebae are stained in the stage before they have aggregated

[4] The culmination process has recently been examined by Raper and Fennell (in press).

[5] The same results were obtained when the amoebae were stained with the red pigment of *Serratia marcescens* using the technique of Raper (1940b).

the suspension picks up the stain uniformly. The cells are then washed free of the dye solution by centrifugation and placed on plain agar. After some hours they aggregate and form migrating masses that are of an even color which is to be expected since the separate cells took up the stain equally. But with the beginning of differentiation, which may appear immediately at the end of aggregation or a few hours later, the anterior region of presumptive stalk remains dark, while the posterior presumptive spore region in a matter of 10 to 15 minutes blanches considerably (fig. 2).[6] The division line between these two regions is invariably clear and sharp. It is, of course, an assumption that this line actually separates pre-spore and pre-stalk cells, but the

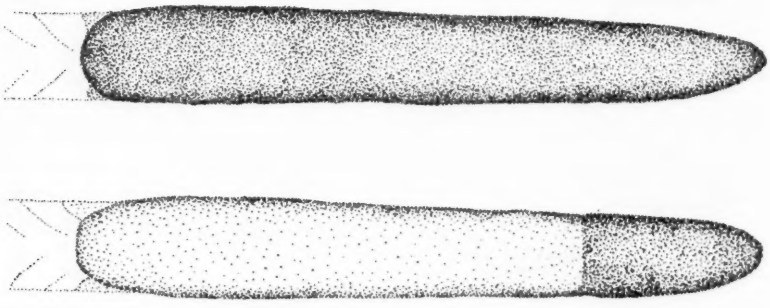

FIGURE 2. Drawing showing how a vitally stained migrating cell mass of uniform coloration (above) will alter to one possessing a dark tip and a light posterior portion (below).

position of the line correlates so perfectly with the separation point of these two tissues that it is hard to imagine it being other than the reflection of the beginning of differentiation.[7] This method may tell one little of the mechanism of differentiation and the separation of the two cell types, but it does show in a striking way how sharply and how suddenly this division may appear. The question now arises as to what extent this division line maintains equal proportions of stalk and spore material in different fruiting bodies.

It must always be kept in mind that starting with aggregation there is no further size increase and that differentiation takes place within a mass of fixed size. The evidence for this is that all food can be eliminated by centrifugation of the amoebae and the cycle is normally completed without the intake of energy. Size then in Dictyostelium is dependent entirely on the mass of cells that comes to one collection point during aggregation, and therefore, it is not surprising that the variation in size among individual aggregates is normally great.

[6] It must be assumed that these color changes do not involve a loss in the dye or a redistribution, but a change in chemical combination in different parts.

[7] If the migration continues for some time the staining becomes irregular and blotchy and is no longer consistent in its pattern except that the very tip will always remain intensely stained.

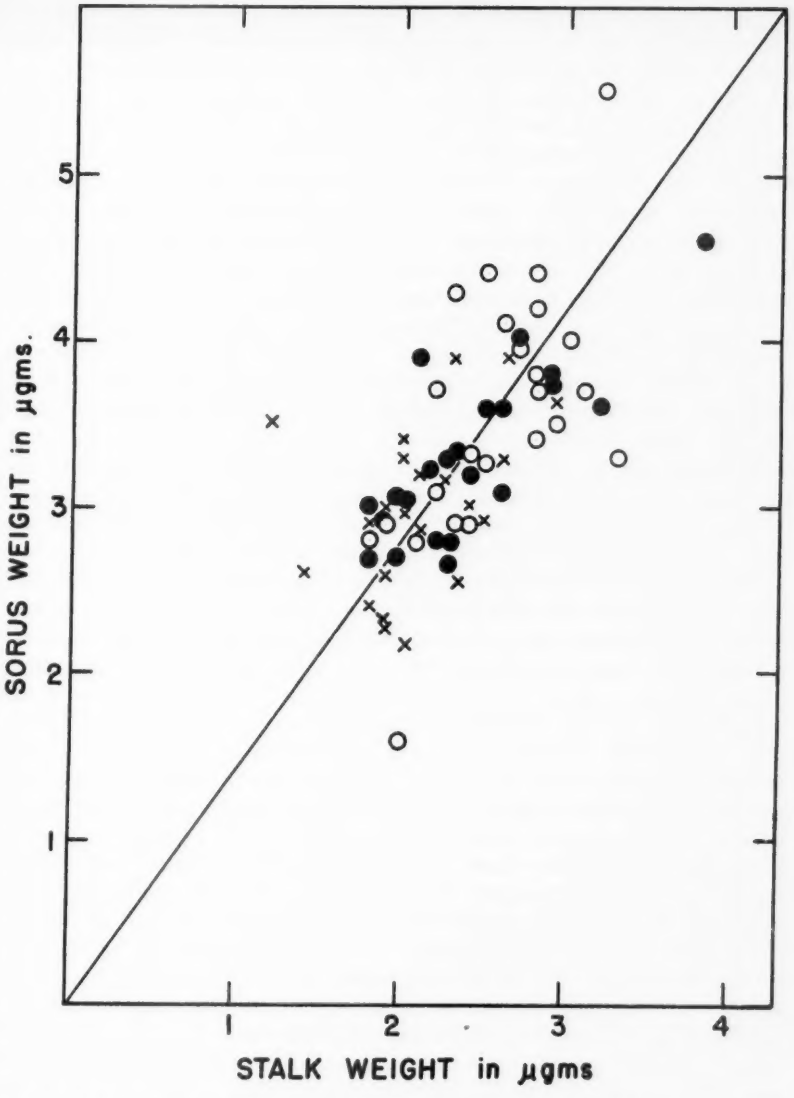


FIGURE 3. A graph in which the dry weight of stalks are plotted against the dry weight of spore masses or sori. Each point indicates a mean value for 30 fruiting bodies from one culture dish. Hollow circles are for those that have fruited at 17° C in the dark; solid dots at 27°C in the dark; crosses at 17°C in the light of a 15 watt "fluorescent" lamp.

If, for a number of different fruiting bodies, the volume of the stalk is plotted against the volume of the spore mass or sorus, then it is seen that with fruiting bodies of different sizes the volume proportions remain approximately constant (Bonner and Slifkin, 1949). The proportions by volume of individuals fruiting under different environmental conditions showed consistent differences, although for any one condition the proportions also remained constant with fruiting bodies of different sizes. In some more recent work I have repeated these experiments using dry weights as a measure. For this a delicate quartz helix balance was used with an average of 30 fruiting bodies to obtain one weight measurement (fig. 3).[8] The curious thing here is that the dry weight proportions remained constant even in different environments, indicating that the volume changes observed previously are most likely caused by great differences in water content arising in the different environments. But the pertinent point to our argument here is that there is a great constancy in the location of the division line between stalk and spore cells.

Let us now examine more closely the equipotential aspect of Dictyostelium and see if the different parts may produce wholes. As a first approach individual cells may be isolated and from these a clone may be grown and its subsequent development followed. A number of workers showed that separate clones invariably gave normal fruiting,[9] but now Sussman (1951) has done a careful and extensive study on just this point. He finds that single spores and single amoebae from any stage of development will give rise to a clone that shows normal development in every respect. Therefore, provided a cell may pass through a period of growth and cell division it is capable of giving rise to stalk and spore cells.

The matter of equipotentiality may also be tested during later stages without any reversion to the vegetative stage. The migrating cell mass is perfectly adapted for isolation experiments and Raper (1940b) has amply explored the many possibilities. He has shown that longitudinal fission is a relatively frequent and normal event and both halves produce normal fruiting bodies. Also occasionally fusions take place naturally, giving one large normal fruiting body, but this occurs only when the anterior ends collide so that the axes of the two cell masses are perfectly aligned.[10] If aggregation streams are cut he again found each portion to give a normal fruiting body. Lastly, if a migrating body is cut transversely all the posterior sections give fruiting bodies of normal appearance and we have corroborated this point by making careful volume measurements of the proportions of various pieces of migrating cell masses (Bonner and Slifkin, 1949). The anterior portion will also give a fruiting body but often it has a disproportionately large stalk, a matter which we will discuss again shortly. From this it is

[8] The sensitivity of the balance was such that 1 mm deflection indicated a weight of 9.12 $\mu$gms. I am indebted to Dr. James H. Gregg (who first used this method on Dictyostelium) for kindly allowing me to benefit from his experience.

[9] Raper (1951). Also D. D. Perkins (unpublished) has had similar results and I have done this successfully a number of times myself.

[10] The matter of polarity in fusion is extremely important (Raper, 1940b; Bonner, 1950).

evident that it is possible to make presumptive spores turn into stalk cells and vice versa, again clearly demonstrating equipotentiality. Recently, I have followed these changes using the vital dye technique described above, and if one makes the transection just at the division line between the blanched posterior section and the dark anterior section, then soon afterward the posterior end will again acquire a dark tip and the anterior section will begin to blanch at its posterior end.

The matter of the proportions upon fruiting of the tip fraction is a bothersome question. I am attempting to find what factors affect the proportions, but at the moment there are no satisfactory answers and considerable confusion and contradiction in the results. It is true, as Raper (1940b) first showed, that often the stalk is large in proportion to the spore mass, indicating that there is sometimes in the very tip some degree of "determination" and that the presumptive stalk cells have to a certain extent a fixed fate. If this is so then it might be that our statement concerning equipotentiality is not so all-inclusive and that the tip, at least in some instances, appears mosaic in character. But the tip has many unusual properties that are lacking in the whole remainder of the cell mass and it may be profitable to examine the tip in more detail.

By the tip I mean approximately the anterior $\frac{1}{20}$ of the cell mass. It is not the whole area of the presumptive stalk cells, but just the anterior-most area. In the first place it was shown previously (Bonner, 1949) that acrasin, the substance responsible for aggregation, was also present in the later stages and that the high point of its production is always at the tip. Furthermore, there is no correlation, no corresponding change, between the acrasin production along the axis of the cell mass and the division line between spore and stalk cells. Also this tip region is in a sense an inductor region, as Raper (1940b) showed, for if a number of tips are grafted laterally on one large migrating cell mass all the tips share the cell mass approximately equally, so that if three new tips are grafted in, four small cell masses will arise. The tip then is an "organization center" although it must be remembered there is no evidence for or against the notion that acrasin is an inductor substance. These properties, then, and possibly also the sensitive photo- and thermo- tropism of the cell mass[11] are properties of the tip alone and do not in any way help us to understand the factors which divide the spore and stalk cells into such stable ratios.

These special tip qualities, however, do again raise the question of whether perhaps there is at the tip a special cell or group of cells (a "queen bee") that is mosaic, that is predetermined at aggregation. After all, the first cells to come into the aggregation center will be those at the tip of the migrating cell mass, and they could have special properties from the very beginning. This hypothesis breaks down for culmination in that during the process of stalk formation the apical cells (which produce acrasin) are constantly changing, constantly becoming trapped, and vacuolate with new cells piling on top of them. But could not this mosaic hypothesis apply for aggregation and migration?

[11] See Bonner, Clarke, Neely and Slifkin (1950).

A few months ago I did some experiments which give some additional information on the nature of the tip. The anterior ends of migrating cell masses stained with a vital dye were grafted into the posterior end of a colorless migrating mass. If the colorless mass is decapitated, as Raper (1940b) had done, then it reorganizes, balls up and attaches itself to the hind end of the anterior colored fragment to form a normal migrating cell mass with a colored anterior end. However, if the colorless host was left intact there was a totally new result: the colored anterior piece slowly moved up the colorless migrating mass so that in a few hours it had achieved a forward position and the whole anterior end was colored (fig. 4). This occurred as the whole mass migrated so that the colored anterior portion travelled at a faster rate than the migrating colorless portion. Furthermore, if a colored posterior piece was grafted into the anterior end it moved backward into the posterior position as migration proceeded. Likewise, anterior portions grafted into anterior positions and posterior portions into posterior positions remained in place.[12] This suggested that the difference between anterior and posterior was one of rate of migration and that the anterior cells were simply there by virtue of being fast. The idea, then, is that migration in the cell mass may represent some sort of Maxwellian velocity distribution and that speed determines position.

The notion that all cells might not move at the same rate is somewhat contrary to the classic idea of migration, for Raper (1940b, 1941) states that if a migrating cell mass half white and half colored is made by grafting, the sharp division line remains throughout migration (6–12 hours) without any conspicuous mixing of the cells. We have repeated these experiments using Nile blue sulphate, neutral red, and using the method involving the red bacterium *Serratia marcescens* and found that, while a distinct division line does remain often for two or so hours of migration, many individual colored cells can be seen to move rather rapidly either forward or backward. In fact, if the cell mass migrates for one or two days it will be evenly colored throughout, but it is quite likely that after such prolonged migration simple physical diffusion also operates.

The evidence then favors the hypothesis that the individual cells each have their own velocity and that the process of migration itself is one in which these different cells are constantly under a process of selection. Only the fastest will be stalk cells and the slowest will be spore cells. This still does not help explain the proportionality that exists between the amount of stalk or spore material, unless one had some critical velocity necessary for stalk-cell formation. But even then it would be hard to see how regulation could take place in a piece isolated by transverse section that gives normal proportions. If there really is a selection here of some

[12] These experiments usually gave rise to abnormal fruiting bodies. A number of controls were run in which no graft was made but a cell mass was lacerated with a needle in a fashion similar to the way it must be in order for the graft to take and similar abnormalities appeared. Those that migrated for long periods after the operation were always normal.

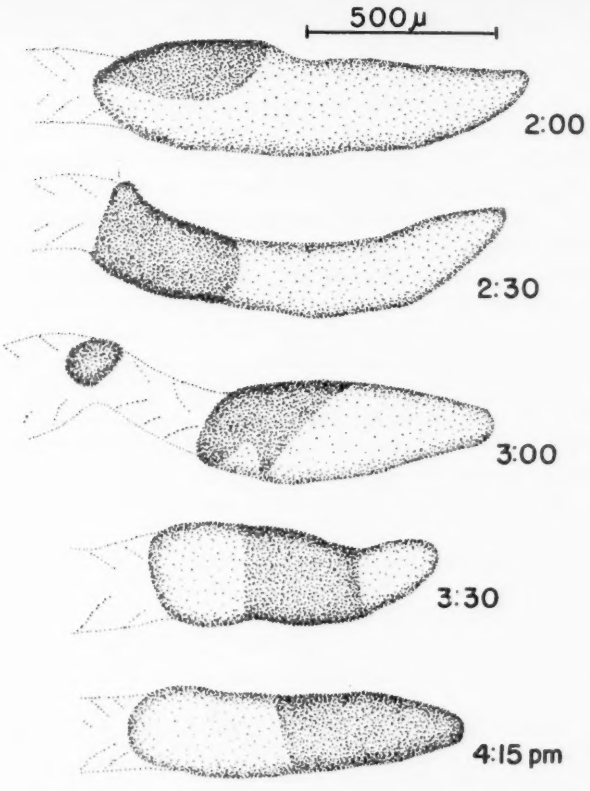


FIGURE 4. Camera lucida drawings showing the rapid forward movement of a colored anterior portion which has been grafted into the posterior region of an intact migrating cell mass. Note that in the middle drawing a piece of the graft was lost.

mosaic character (reflected in the speed of the amoebae) then it should be possible to test this hypothesis by artificial selection.

This was done by taking a migrating cell mass and cutting off a small section of the anterior end. We allowed this anterior portion to fruit and its spores were used to start an $F_1$ generation. When a migrating sausage was produced in this $F_1$ then it was also isolated and its anterior end separated just as before. This was repeated until the $F_{10}$ generation and each of the generations produced normal fruiting bodies which strongly suggests that no selection has taken place. Both posterior and middle fractions of migrating masses were also selected for in the same way for five generations and again no change in the fruiting bodies before and after this repeated selection could be detected. So it would seem, from the present evidence, that even though there be differences in rates among amoebae in the mass,

this does not reflect any mosaic basis of differentiation and the parts of the cell mass are equipotential.

If we now review the salient facts that have been presented we see that in this aggregate of cells there is a division line between the two cell types and that this division line is proportional irrespective of the number of cells in the aggregate. This proportionality cannot be at the moment understood in terms of the tip for the peculiar activity of that apical region, which includes induction and the production of acrasin, does not coincide geographically with the division line between spore and stalk cells. Each fragment of the cell mass appears equipotential and will produce (if it lacks it) a new division line, which may involve stalk cells changing to spore cells and vice versa, and a new tip region. The most stable entity that cannot be altered is the polarity, for the antero-posterior axis remains fixed and cannot be altered experimentally (Bonner, 1950).

When one looks over these facts as I have presented them here it is alarming to realize that even though we know some details of the development of Dictyostelium, and a great many details concerning the regulatory development of other animals, we understand no better than did Driesch the explanation of a harmonious equipotential system, and yet a half century of embryology has elapsed. It may be that we no longer find entelechies a temptation, but our substitutes are poor indeed. All we can say is that there are physical analogies that might help us and this thought keeps us confident that ultimately physics and chemistry will not fail us in an explanation. And the only thing we may pretend that our glance at Dictyostelium has done is to expose, to dissect out the problem so that possibly it may be seen more distinctly—so that possibly it may keep us from forgetting that one of the most important problems of morphogenesis is still unsolved and still with us.

## SUMMARY

The following original experimental material is presented in this paper.

1. If the separate amoebae of *Dictyostelium discoideum* are stained with a vital dye the migrating cell mass will first be of a uniform color but later the anterior portion will remain dark and the posterior portion will blanch. The sharp division line corresponds to the position of the division line between presumptive stalk and spore cells. If such a cell mass is cut transversely at the division line, the posterior portion will again become dark at the tip, and the anterior portion will blanch at its posterior end.

2. If the dry weight of the stalk material is plotted against the dry weight of the spore mass a strict proportionality exists even under different environmental conditions.

3. By marking groups of cells with vital dyes and by grafting them in different regions of migrating cell masses it was shown that anterior cells move at a relatively faster rate than posterior cells; if marked anterior cells were placed in the posterior portion, in the course of a few hours they moved up to the anterior end as the whole mass migrated. Likewise, marked pos-

terior cells placed in the anterior end would fall back to a posterior position.

4. It could be seen directly, using vital dyes, that individual cells or groups of cells moved faster or slower than their neighbors, and that movement during migration did not involve a uniform speed of all cells.

5. If anterior fractions of migrating cell masses were each independently selected for in repeated generations, by allowing them to fruit, sowing their spores, and isolating an anterior fraction in the next generation, after a number of such generations the fruiting bodies produced were similar and normal. This was also true for middle and posterior fractions indicating that there is no mosaic segregation taking place during migration.

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# STEREOSCOPIC STUDIES OF CELLS AND VIRUSES IN THE ELECTRON MICROSCOPE[1,2]

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The electron microscope has two big advantages over its older cousin, the light microscope. The first of these is its high resolution, 0.002 μ as compared to a resolution of only 0.2 μ for the light microscope. By dint of this resolution the electron microscope has already given a number of indications on an almost molecular scale of how certain cellular constituents and products are arranged. The second advantage, which is less well known, is the great depth of focus of the electron microscope, which, being about ten times that of the light microscope, makes stereoscopic electron microscopy possible. However, until recently the stereoscopic determination of form has been applied only to certain rugged biological elements such as butterfly scales and diatom shells which can withstand drying.

To the biologist the principal disadvantage of the electron microscope is this fact. Most of his specimens, containing water as an essential part, must be freed of all volatile material before they can be studied in the high vacuum of the instrument. The electron microscope permits the study of only dry and dead objects. In view of this limitation we should be well content if we could only dry our material in such a way that its form is not distorted.

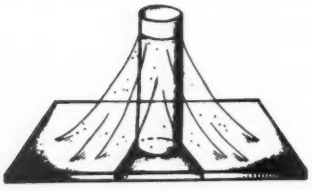
The primary source of distortion during air-drying is the relatively enormous force exerted by surface tension as water evaporates from the specimen. The magnitude of these forces may be appreciated by reference to figures 1 and 2 where the stresses acting on two types of microscopic structure are estimated. It may be seen from figure 1 that the column 0.02 μ in diameter must be able to withstand a stress of 2000 pounds per square inch if it is not to collapse, while the 0.02 μ cord bridging the 2 μ gap shown in figure 2 is subject to more than 300 tons per square inch!

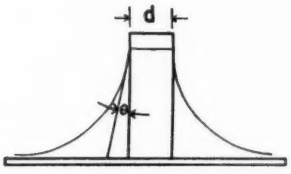
Such stresses may be avoided by imbedding the material in non-volatile wax and cutting sections which are thin enough for study in the electron microscope. But the imbedding agent reduces contrast in the electron image. Furthermore, only small parts of cells are visible in a single section since

[1] Presented at the Symposium of the American Society of Naturalists on Patterns of Cellular Organization, Minneapolis, Minnesota, September 12, 1951.

[2] This work was supported by a contract between the Office of Naval Research, Department of the Navy and the University of Pennsylvania (NR-135-197), Physical, Chemical and Biophysical Characterization of Viruses and Virus Systems. Reproduction in whole or in part is permitted for any purpose of the United States Government. The technical assistance of Mr. Carl F. Oster is gratefully acknowledged. Dr. Warner E. Love helped in the preparation of the ghosts of human red cells shown in figures 4 and 5.

SURFACE TENSION ACTING ON A SMALL COLUMN






Total force on column: $F = \pi d \gamma \cos\theta$

Cross sectional area: $A = \frac{\pi}{4} d^2$

Stress on column: $S = \frac{F}{A} = \frac{4\gamma \cos\theta}{d}$

for:
$\gamma_{H_2O}$ = 70 DYNES/CM.
$\theta = 0°$
$d = 200\,\text{Å}$
$S = 2000$ LB./IN.$^2$

FIGURE 1. A calculation of the force, $F$, produced by surface tension, $\gamma$, acting on a small cylindrical column of diameter, $d$. The stress on the column, $S$, turns out to be inversely proportional to the diameter. As a result a column 0.02 $\mu$ thick and wet by water ($\gamma = 70$ dynes/cm, $\theta = 0°$) must be able to withstand a stress of 2000 pounds per square inch if it is not to be deformed or broken during evaporation of the film of water surrounding it.

it must be thinner than 0.2 $\mu$ to permit the electron beam to penetrate it. Dr. Robert D. Boche and I tried freeze-drying some cells ten years ago and obtained pictures of chromosomes by this method which showed less distortion than those of air-dried material, but which were still somewhat disappointing. The specimens seemed to be brittle at the low temperature. Theoretically, too, freeze-drying is not very satisfactory since in this procedure potentially disruptive phase boundaries pass through the specimen twice; first, the solid-liquid boundary on freezing the water, and second, the solid-vapor boundary on subliming the ice.

In general it may be said that the passage of any phase boundary through a specimen will have a tendency to deform it because of (1) the inherent tendency of surfaces to adsorb materials which will reduce their specific surface energies, and (2) their tendency to deform their shapes in such a way that they minimize their surface areas. Moving surfaces therefore tend to carry specimens with them and so deform them.

It is possible to dry specimens without having any phase boundaries pass through them by using a method which was found to have been invented by S. S. Kistler (1932) for preparing aerogels. Kistler substituted alcohol for the water in a gel and then, with the gel in a bomb almost full of alcohol, he raised the temperature to 250°C, well above the critical point of the alcohol where the liquid changed imperceptibly into a gas. This gas he let escape at the elevated temperature. Then when he had substituted air for the residual alcohol vapor he had prepared a so-called aerogel in which air replaced those microscopic spaces in the gel formerly occupied by water.

For the preparation of specimens we felt that a temperature of 250°C might be too severe for biological materials. We therefore modified Kistler's procedure by fixing specimens in osmic acid vapor and then replacing the water by each of a series of miscible liquids in turn (alcohol, amyl acetate, and liquid carbon dioxide), the last in the series, carbon dioxide under pressure in a bomb, being a liquid with a critical point of only 31°C. Raising the temperature of the liquid to 45°C converts it imperceptibly to a gas which is allowed to escape. When the specimen is removed from the bomb and placed in the vacuum of the electron microscope even the residual gas is removed. The interstices of the specimen are empty and uncollapsed by the surface tension of the liquid which had filled them (Anderson, 1951).

In some cases the improvement given by this "critical point method," as we have called it, is apparent even in single pictures. For example, figure 3A is an electron micrograph of a "mitochondrial fraction" from pea seedlings dried in air, while figure 3B is the same material dried by the critical point method. The difference is striking: while the former has the appearance of an amorphous mass of debris, the latter consists of well-defined membranes many of which contain internal structures.

From stereoscopic pictures of a specimen like this the shapes of such objects can be determined in detail. A picture of the specimen is taken, the specimen is tilted through a definite angle, and another picture is taken.

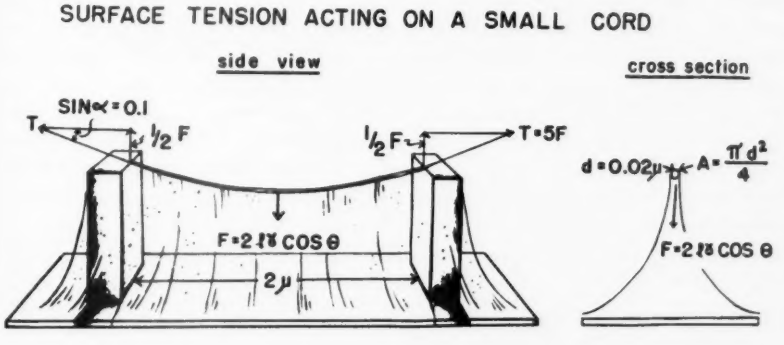



FIGURE 2. A calculation of the force of surface tension acting on a cord of diameter, *d*, spanning a gap, *l*, cm wide, and at its ends making an angle ∝ with the horizontal. A cord 0.02 μ thick stretched across a 2 μ gap would have to be stronger than piano wire if it were not to break during evaporation of the film of water which it supports.

The two pictures form a stereoscopic pair which, mounted side by side, can be viewed with the eyes and fused to give the impression of depth; or they may be measured with a micrometer to yield relative elevations much as is done with pictures obtained in aerial mapping.

To gain experience with the method and discover what new types of artifacts might arise through its use, we have studied a number of biological materials. One of the best test objects is hemolysed human red cells like those shown in the stereoscopic pairs of figures 4 and 5. The cells have retained their spherical shapes. In the same preparation one can find cells with very smooth surfaces and others with crenated surfaces but with no internal structures which may have caused the crenations. Some of the cells in a hemolysed preparation have within them extremely fine strands of an as yet unidentified material as may be seen in the cell of figure 5.

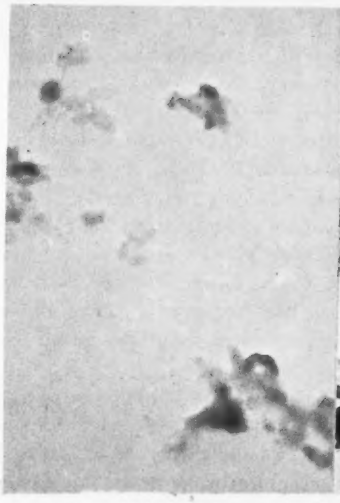
In figure 6 are shown part of an extruded trichocyst of *Paramecium aurelia* exhibiting characteristic cross striations and a number of spirilla with their flagellae stretched across many microns. The helical shapes of the bacteria are well preserved.

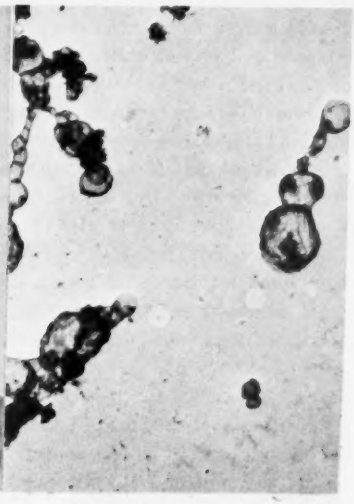
This work was undertaken primarily for the purpose of studying the reactions of bacteriophages with their host cells. We had felt that artifacts introduced by the old air-drying techniques had given misleading pictures like that of a mixture of T2 phage and host cells shown in figure 7. It is seen that the periphery of the almost completely flattened cell is lined with the tadpole-shaped virus particles, but that there are none lying on top of the cell. This made us suspect that most of the particles had not been adsorbed on the cells at all but had been pushed up to the edge of the cell under the surface of the evaporating water as it receded toward the cell. The whole field of figure 7 is remarkably flat compared to fields of specimens prepared by the critical point method. When prepared by this method, specimens like those shown in figures 8 and 9, in which little physiological adsorption had occurred, indeed showed few particles touching the bacteria; specimens in which adsorption was known to have occurred showed cells like that of figure 11 which were coated with particles attached by their tails, reminiscent of pins stuck in a pincushion. As seen in figure 12 the virus particles attach to ghosts of the host cells in the same way, i.e. by their tails. As yet we do not know the significance of the fine strands of material within some of the ghosts, but the larger ovoid bodies seem to represent shrunken residues of the cytoplasm which resists the enzymatic digestion employed in their preparation.

The new drying method gives us a new concept of the shapes of the virus particles, too, for the particles standing on their tails as seen in figure 10 have the cross section of a regular hexagon. Seen from the side the heads present a hexagonal profile also, but with the two sides parallel to the tail slightly longer than the other four sides. The related phages T2 and T6 have morphologies very similar to that of T4.

In figure 13 is shown an unrelated phage, T5, which is active on this same strain of *E. coli*. Earlier air-dried specimens of T5 had shown (Ander-

3A





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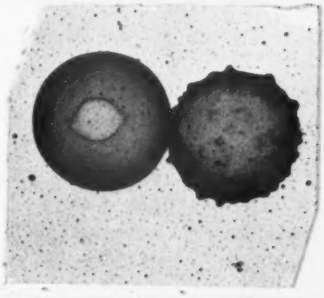

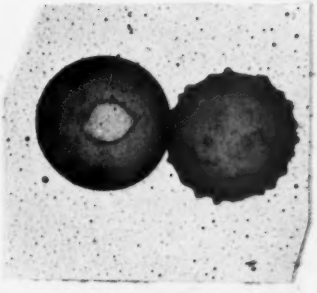

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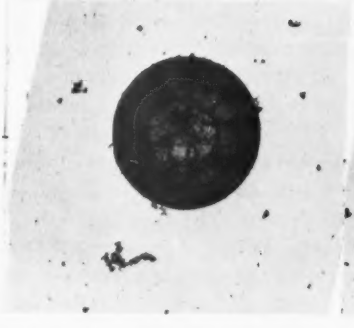


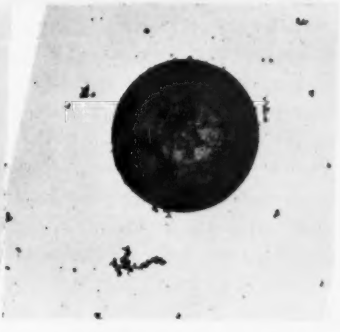


FIGURE 3. A mitochondrial fraction from pea seedlings prepared by Dr. Helen A. Stafford (1951). Specimen A was dried in air, while that shown in B was prepared by the critical point method. Magnification of A × 8500, of B × 12 400.

FIGURE 4. A stereoscopic pair of electron micrographs of washed human red cells hemolysed by the addition of water to the physiological saline solution in which they had been suspended and then fixed and dried by the critical point method. Magnification × 4750, stereoscopic angle 12°.

FIGURE 5. A hemolysed red cell prepared like those shown in Figure 4, but containing within it some extremely fine strands of an unidentified material. Magnification × 5350, stereoscopic angle 12°.

son, 1946) flattened and almost empty heads to which a ragged, partially disintegrated tail was attached. The new pictures show that T5 has a well-defined tail and a head which, like those of T2, T4, and T6, presents an almost crystalline appearance. However, the head of T5 is more nearly spherical and the tail is longer than those of the even-numbered phages.

A large part of the internal structure of the heads of the even-numbered phages can be removed by osmotic shock: suddenly reducing the concentration of a solute like NaCl or glycerine in the environment from 3M to a low value. This produces what may be termed a "ghost" of the phage particle: an empty head membrane to which the tail remains attached. T5 on the other hand seems to resist osmotic shock, but, as shown in figure 14, ghosts of T5 are produced when this phage is inactivated by gently heating in the absence of calcium ion.

Apparently then, all four phages, T2, T4, T5, and T6, consist of at least three identifiable structures: 1) the tail whose end contains the apparatus for specific adsorption on the host cell; 2) a head membrane which is firmly attached to the tail; and 3) an internal structure in the head which is easily removed in one manner or another, but which, together with the head membrane, presents the appearance of a tiny crystal when dried by the critical point method.

In conclusion it might be well to introduce some of the artifacts that the method reveals. So far at least, these artifacts may be considered to arise through surface tension, not that of the ambient liquid of course, for it has been eliminated, but through the surface tension of the specimen itself and the tendency of the specimen to reduce its own surface energy. For example, specimens like the red cell ghosts seem to have shrunk in size. This doubtless reflects a tendency of the specimen to reduce its total surface area by collapsing the holes of molecular dimensions left in it after the removal of water, salts and lipids. Therefore we cannot place too much reliance on the observed sizes of objects from which such extractions may have occurred. Nor can great stress be placed on the observed shapes if the object could have become warped by collapsing unequal concentrations of holes in its various parts.

In one way the surface tension of the specimen is of considerable help. In a vacuum the specimen must be in contact with some solid object or it would fall out of the field. At a point of contact the specimen will tend to reduce surface energy by fusing its surface with that of the support, Brownian motion bringing new points into contact until the energies necessary to continue the process further become prohibitive. Thus the flagellae in figure 6 tend to lie in contact with the supporting film and, where they leave it,

FIGURE 8. *Escherichia coli* strain B mixed with T4 bacteriophage without added tryptophan to promote adsorption. The mixture stood for 1 min. at 32°C before a specimen was prepared by the critical point method. The field is quite deep because of the elimination of surface tension forces. The field has been shadow-cast with gold, but the bacteria do not cast shadows because they lay on the side of the formvar membrane facing away from the source of the gold. Magnification × 20 000, stereoscopic angle 12°.

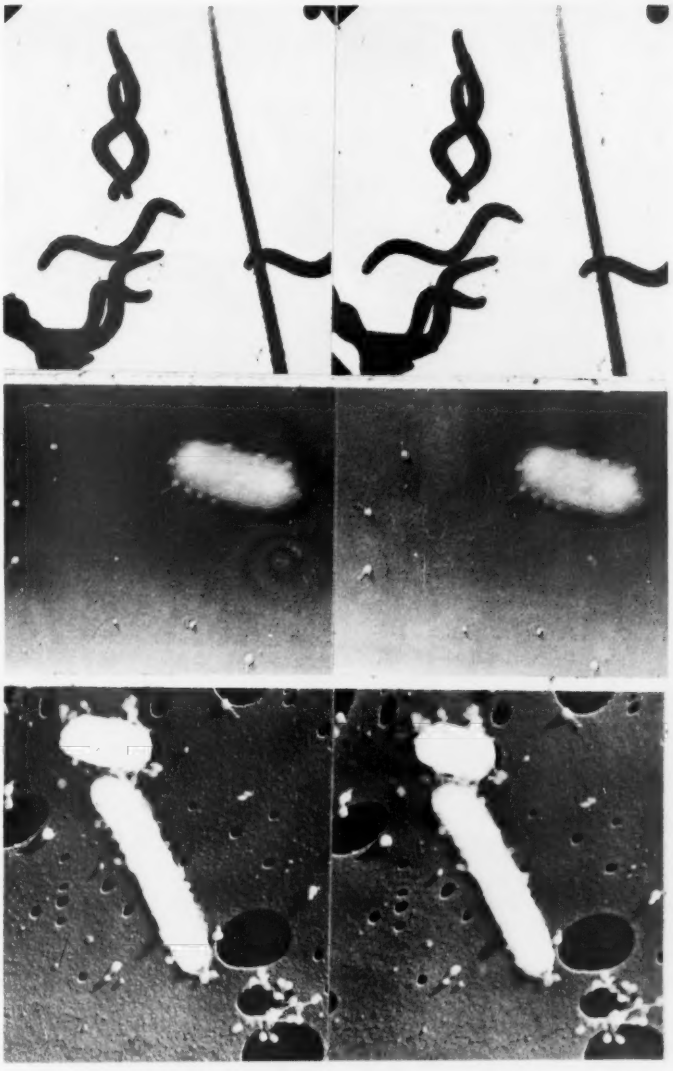
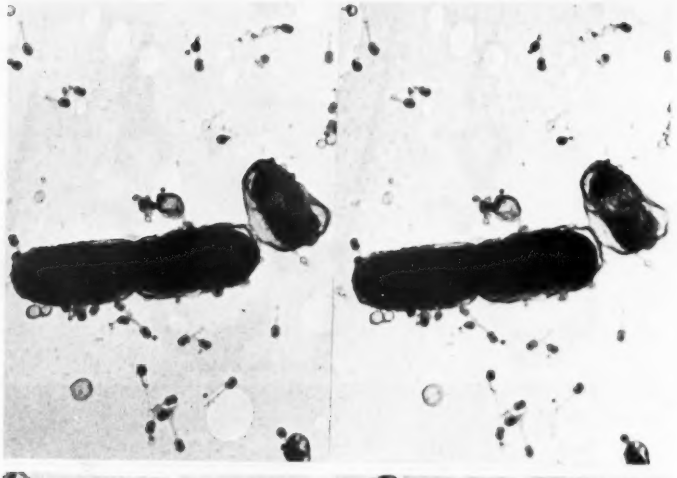


FIGURE 6. An extruded trichocyst of *Paramecium aurelia* together with a number of bacteria (spirilla) which were present in the culture prepared by the critical point method. This specimen was micrographed with the Phillips electron microscope at the Rockefeller Institute for Medical Research through the kindness of Dr. K. R. Porter. Magnification × 10 800, stereoscopic angle 10°.

FIGURE 7. *Escherichia coli* strain B mixed with T2 bacteriophage and given 30 sec. for adsorption of the phage on the host cells at 18° C. A specimen was then mounted on a specimen screen, washed in water to remove salts and dried in air. The specimen was shadow-cast with gold. Very little depth can be seen in this specimen because the surface tension of the water has flattened it. Magnification × 13 100, stereoscopic angle 12°.

9



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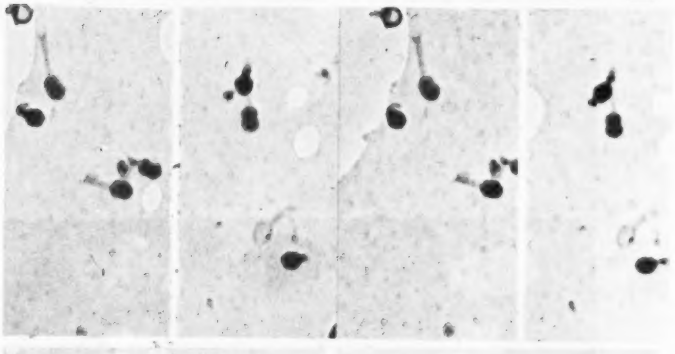

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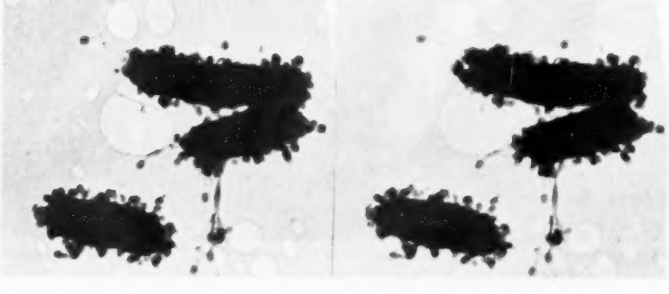
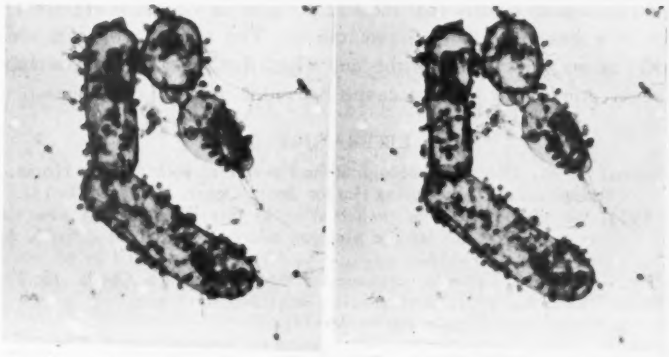


FIGURE 9. The same specimen but a different field than that shown in Figure 8 micrographed before shadow-casting. Magnification × 22 000, stereoscopic angle 12°.

FIGURE 10. Parts of the field of Figure 9 reproduced at higher magnification to show the form of the T4 virus particles after they have been dried by the critical point method. Magnification × 43 000, stereoscopic angle 12°.

FIGURE 11. A specimen prepared as that of Figure 8, but in which tryptophan had been added to the mixture of *E. coli* and T4 to promote the adsorption of the virus on the host cells. It may be seen that after one minute at 32°C the cells are loaded with phage particles adhering by their tails. Magnification × 22 000, stereoscopic angle 12°.

12



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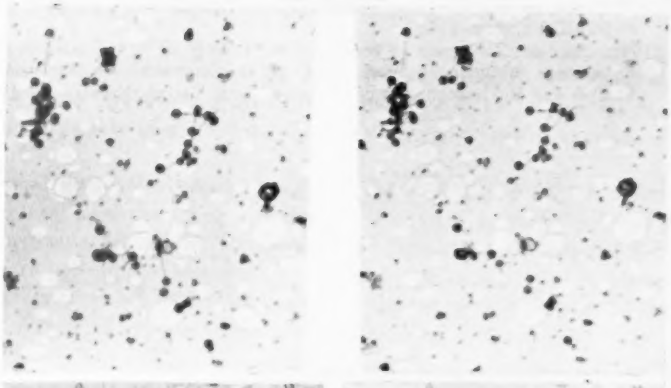

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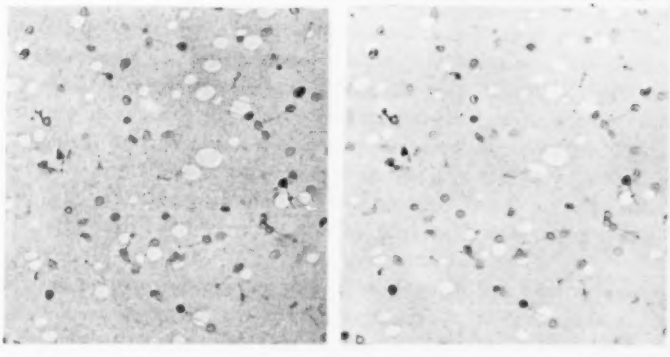

FIGURE 12. Ghosts of *E. coli* strain B (Weidel, 1950, 1951) on which are adsorbed particles of the bacteriophage T2. Magnification × 22 000, stereoscopic angle 12°.

FIGURE 13. The bacteriophage T5 prepared by Mr. Gordon Lark and dried by the critical point method. The well-defined tails and the almost crystalline shapes of the heads are apparent. Magnification × 23 700, stereoscopic angle 12°.

FIGURE 14. Phage T5 which had been inactivated by heating in the presence of citrate to reduce the concentration of calcium ion which otherwise would have rendered it stable to the gentle heating which was employed. The sample prepared by Mr. Lark was dried by the critical point method. Magnification × 25 400, stereoscopic angle 12°.

are stretched so tightly that their amplitudes of vibration are not detectible. This is a most fortunate circumstance. The very mechanism which tends to distort our specimens is the one which fashions for them a support sufficiently sturdy that in most cases Brownian motion is not seen.

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# CYTOLOGICAL OBSERVATIONS CONCERNING INVERSION AND TRANSLOCATION IN THE HOUSE MOUSE[1]

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The existence of balanced lethal lines in the tailless house mouse, such as $Tt^0$ and $Tt^1$ (Chesley and Dunn, 1936), and the proof that two of the lethals, $t^0$ and $t^1$, prevent recombination in the region of $T$ (Dunn and Caspari, 1945), suggested that $t^0$ and $t^1$ are associated with inverted sections of Chromosome IX. The discovery of several additional mutations of the "$t$" type, each of which fails to show recombination with $T$ (Dunn and Gluecksohn-Schoenheimer, 1950), has raised the question whether all "$t$" mutations contain the same inversions or whether, since the new "$t$" mutations have arisen spontaneously from $T/t^1$ stocks, they represent new inversions induced by $t^1$.

Cytological study of spermatogenesis in the $T/t^0$ (Line A), $T/t^1$ (Line 29), and control males was begun in the fall of 1949. The technique finally chosen involved the use of non-decolorized basic fuchsin applied after hydrolysis to squash preparations, similar to the procedure used by Slizynski (1949). It appeared to give stronger staining of meiotic prophases than the Feulgen technique. The essential steps were as follows:

## METHODS

The testes of 6–8 weeks old mice are removed immediately after killing, split and teased apart in "warm-blooded" Ringer's solution until most of the semeniferous tubules float free. For each preparation, a 5–10 mm. length of tubule is placed in a drop of aceto-carmine on an albumenized slide and squashed by quickly dropping a clean slide on top of it. Taking care that there is no untoward slipping, the material is evenly squashed by applying a rolling pressure with the thumb or a cork. The two slides which now stick together are heated very gently by passing 5 or 6 times over the flame of an alcohol lamp.

The two-slide preparation is then placed on edge in San Felice solution in a staining dish in which the slides separate in 10–20 minutes, the squashed material remaining on the albumenized slide. This slide is

[1]Studies carried out at the Biological Laboratories, Harvard University, and, during the summer of 1950, at the Nevis Biological Station of Columbia University, with the advice and criticism of Dr. Gerhard Sander and Professor L. C. Dunn. Grateful acknowledgements are made to Professors A. B. Dawson and Karl Sax of Harvard University.

[2]Since Mr. Jaffe has been recalled to active duty in the U. S. Navy and his work on this problem consequently interrupted, this condensation of a longer illustrated report has been prepared to serve as a preliminary note pending Mr. Jaffe's resumption of study.

L. C. DUNN

rinsed in distilled water, placed for 7 minutes in normal HCl at 60° C., rinsed again, and then placed in 0.5 per cent aqueous basic fuchsin until adequately stained, usually for 5–15 minutes. The slide is then rinsed and bleached in $SO_2$ water, dehydrated through an ethanol or ethanol-butanol series and mounted in Gum Damar.

## RESULTS

The results of a search for chromatid bridges and acentric fragments at Anaphase I and of persistent acentric fragments in Anaphase II are shown in table I.

TABLE 1

CHROMOSOMAL ABERRATIONS IN ANAPHASES I AND II INDICATING THE PRESENCE OF INVERSIONS

| Animal | Genotype | Anaphase I | | Anaphase II | |
|---|---|---|---|---|---|
| | | Aberrant | Normal | Aberrant | Normal |
| 1 | $T/t^1$ | 1* | 90 | 0 | 18 |
| 2 | $T/t^0$ | 0 | 74 | 2 | 131 |
| 3 | $T/t^0$ | 1 | 21 | 3 | 178 |
| 4 | +/+ | 0 | 82 | 0 | 32 |
| 5 | +/+ | 0 | 97 | 0 | 21 |
| 6 | +/+ | 0 | 28 | 0 | 47 |
| 7 | +/+ | 0 | 23 | 0 | 28 |

*Doubtful figure; bridge is present, but what appears to be the fragment may be a broken chromosome.

Only one bridge and fragment was found at Anaphase I in the heterozygotes and none in the controls. The five fragments at Anaphase II may have been derived from Anaphase I fragments or in other ways.

In the course of study other irregularities were noted which could be distinguished from the true bridge-and-fragment configuration. Pseudo-bridges at Anaphase I were found in 2.5 per cent of anaphases of both mutant and control animals. These appeared to be due to sticking of corresponding arms of separating bivalents. No accompanying fragments were ever seen. Pseudo-bridges were noted by Schultz and St. Lawrence (1949), and Therman and Timonen (1951) in human chromosomes. Painter (1923, 1927) observed that the separating X and Y in man and in the mouse often appeared to form a bridge. It has not yet been determined whether pseudo-bridges in the mouse are confined to the XY complex.

The analysis of Anaphase I was further complicated by lack of synchronization in separation of chromosomes so that laggards were rather frequently found (ca. 10–20 per cent). Similar experiences have been recorded by Schultz and St. Lawrence (1949) and Painter (1923), but Makino (1941) believes the separation of sister chromosomes in the mouse to be synchronous and explains laggards as fixation artifacts.

In view of these complications only bridges with acentric fragments were considered indicative of the presence of heterozygous inversion. The

few reports of inversions in mammals (Koller, 1936; Crew and Koller, 1939; Slifer and Beams, 1949) have been viewed critically by other cytologists (Schultz and St. Lawrence, 1949) because of the difficulties of distinguishing true inversion bridges from pseudo-bridges.

The data of table 1 do not support the hypothesis that the balanced tailless lines $T/t^0$ and $T/t^1$ have a large inversion, since crossing over in such an inversion should lead to an appreciable frequency of bridge-and-fragment configurations. A small inversion is not ruled out and might satisfy the genetical data since the extent of suppression of crossing over in Chromosome IX is not known, except for the short interval *T-Ki*, about four crossover units according to Dunn and Caspari (1945).

## EVIDENCE OF TRANSLOCATION

In an attempt to produce mutations with which to find markers for Chromosome IX a number of normal males were X-rayed with from 300r to 1,000r by Professor L. C. Dunn and studied genetically by him and Mr. Walter C. Morgan. In addition to several new recessives, seven semi-sterile lines were isolated similar to those described by Snell (1941). Thus far translocations have been found in spermatogenesis in the three semi-sterile lines which have been adequately examined. These appear as rings of 4 at diakinesis but have also been seen in pachytene, diplotene, and metaphase. In one line (22746) the interchange appears to involve two of the larger chromosomes; that in another line (22757) seems to involve a large and a medium-sized chromosome, showing both rings and chains of four; while the third line (665) involved a medium-sized and a small chromosome, showing less clear-cut rings and frequent chains of four. Experiments were undertaken to determine whether any of the chromosomes involved was Chromosome IX, but these have not yet given conclusive results.

## SUMMARY

1. Balanced lethal lines of tailless mice containing a crossover suppressor have been examined cytogenetically. A low frequency of chromatid bridges and fragments in anaphase indicates that if inversion is present it is very small.

2. Pseudo-bridges and lagging chromosomes were found rather frequently in spermatogenesis of both tailless and control animals.

3. Chromosome rings indicating segmental interchanges have been found in spermatogenesis of semi-sterile males, belonging to three different semi-sterile lines.

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# THE EFFECT OF THE BRONZE LOCUS ON ANTHOCYANIN FORMATION IN MAIZE

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The interactions of loci affecting pigment formation in plant and aleurone tissue have been extensively studied in the past by students of maize genetics. The phenotypes produced by different combinations of dominant and recessive alleles of the loci essential for anthocyanin and anthoxanthin production are listed in summary form in Emerson et al. (1935). Five loci, $A_1$, $A_2$, *B*, *Pl* and *R*, are concerned with plant color while the $A_1$, $A_2$, *C* and *R* loci are basic to the formation of aleurone color. A number of modifying genes are known which affect the kind or amount of pigment produced rather than its primary formation. For example, the *Pr* locus determines whether the aleurone color is red or purple. Since the genic interactions in plant and aleurone color have been so clearly elucidated and since many of the differences produced by the substitution of a recessive allele for a dominant are so strikingly different, attention has been given in recent years to the biosynthetic steps involved in the production of anthocyanins and anthoxanthins in maize. If the effects of allelic differences are reflected in simple modifications of the molecular structure of the pigment molecule it may be possible to approach the problem of gene action and also to ascertain the biosynthetic steps involved in a series of sequential reactions. Although the recent studies by Laughnan (1948, 1950, 1951) indicate that pigment biosynthesis in maize is more complex than anticipated, nevertheless a joint genetical and chemical attack on pigment formation in maize promises to be a fruitful and rewarding endeavor. Added interest in this problem has come through the discovery of a new locus with profound effects on pigment formation. The purpose of this note is to describe the interaction of this new locus with the other loci involved in plant and aleurone color and to give its linkage relations. The recessive mutant allele arose in the inbred line Hy and was called to the writer's attention by Dr. M. T. Jenkins.

As the data in table 1 show, the bronze locus (dominant allele *Bz*, recessive *bz*) is in chromosome 9 approximately two map units proximal to the shrunken-1 locus. McClintock (1931) has shown that the *C*, *sh*, and *wx* loci all lie in the short arm of chromosome 9 with *wx* nearest to the centromere. These three loci are concerned with aleurone color, endosperm development and type of endosperm starch, respectively, so the inclusion of the *bz* gene in this constellation of useful genetic markers makes the short arm of 9 unusually valuable for cytogenetical investigations.

Seeds of $A_1\ A_2\ C\ R$ constitution and possessing the *Bz* allele have a purple aleurone color with *Pr* and a red color if recessive *pr* is homozygous. When the *bz* allele is made homozygous, kernels of $A_1\ A_2\ C\ R\ Pr\ bz$ constitution have a strong bronze-like coloration and $A_1\ A_2\ C\ R\ pr\ bz$ seeds have a lighter bronze color. The pigment or pigments giving the bronze phenotypes are located in the aleurone grains as is the case for purple or red colored seeds. No pigment is found in the cell walls of the aleurone layer.

Plants of $A_1\ A_2\ B\ Pl\ Bz$ constitution have the customary deep purple coloration in the culm, leaves and tassel while the substitution of the *bz*

TABLE 1

BACKCROSS DATA FROM THE CROSS OF *C Sh bz / c sh Bz* INDIVIDUALS BY *c sh bz* POLLEN SHOWING THE LINKAGE RELATIONS OF THE *bz* GENE WITH THE *C* AND *sh* LOCI IN CHROMOSOME 9. IN KERNELS CARRYING THE DOMINANT *C* ALLELE THE CLASSIFICATION FOR *Bz* AND *bz* WAS MADE BY ALEURONE COLOR WHILE PLANT COLOR WAS USED FOR *Bz* AND *bz* SCORING IN SEEDS WITH RECESSIVE *c* AND COLORLESS ALEURONE.

| (0) | (0) | (1) | (1) | (2) | (2) | (1–2) | (1–2) |
|---|---|---|---|---|---|---|---|
| *C* | *c* | *C* | *c* | *C* | *c* | *C* | *c* |
| *Sh* | *sh* | *sh* | *Sh* | *Sh* | *sh* | *sh* | *Sh* |
| *bz* | *Bz* | *Bz* | *bz* | *Bz* | *bz* | *bz* | *Bz* |
| 1396 | 1354 | 76 | 65 | 15 | 31 | 0 | 0 |

*C* – *Sh* = 4.8 per cent recombination
*Sh* – *Bz* = 1.6 per cent recombination
*C* – *Bz* = 6.4 per cent recombination

Linear order of genes in short arm of chromosome 9 is
Dt – $yg_2$ – C – sh – bz – bp – wx – centromere.

allele produces a deep rust-colored pigmentation in the same tissues. In *Bz* plants, no brown pigments are present and the anthocyanin is confined to the vacuoles. However, *bz* plants have deeply-colored, brown cell walls and greatly reduced amounts of anthocyanin in the vacuole. In some cells cytoplasmic granules with a reddish hue are observed. The pigment of $A_1\ A_2\ B\ Pl\ bz$ plants is produced in darkness as is that of *Bz* individuals.

Plants of $A_1\ A_2\ B\ pl\ Bz$ constitution develop a red pigmentation (sun red), rather than the purple produced in the presence of *Pl*, in those tissues which are exposed to light. When the *bz* allele is substituted for *Bz* in individuals of the above genotype a bronze plant color results which is also a sun color in that no pigment is formed in local darkness. In sun red plants *(Bz)* no brown pigments are found and the anthocyanins are confined to the cell vacuoles; in *bz* plants the cell walls are brown in color. As in $A_1\ A_2\ B\ Pl\ bz$ plants a much reduced amount of anthocyanin is present.

The coloration of $A_1\ A_2\ b\ Pl\ Bz$ (dilute purple) and $A_1\ A_2\ b\ pl\ Bz$ (dilute sun red) plants is bronze when the *bz* allele is homozygous. The cell walls in the pigmented regions of the *bz* plants are brown. Traces of anthocyanin were observed in the vacuoles of some cells.

In addition to the above studies on the interaction of *bz* with other plant color loci, Laughnan (1951) found that the pigmentation of $A_1$ $a_2$ *B Pl Bz* plants was visibly different from that of $A_1$ $a_2$ *B Pl bz* individuals.

In summary it may be stated that the *Bz* locus does not determine whether or not plant and aleurone pigments will be formed but does exert a profound influence on the kind of pigments produced and in some instances on their location within the cell. No effect of the *Bz* locus has been found on pericarp color.

McClintock (1951) has made extensive use of the *Bz* locus in her remarkable studies on mutable loci. A number of her findings are worthy of mention here. Aleurone cells homozygous deficient for the *Bz* locus have a bronze phenotype similar to that produced by the recessive *bz* allele. Kernels of *C bz wz ds/C bz wx ds/I Bz Wx Ds* constitution have a colorless aleurone (due to the action of the dominant *I* gene which is an allele of *C*) and endosperm starch which stains blue with I-KI. However, in some of the mitoses of the developing kernel chromosome breakage occurs at the *Ds* locus if the Activator locus (*Ac*) is present. That portion of the short arm of chromosome 9 distal to the *Ds* locus forms an acentric fragment and is eliminated. Sectors arising from cells in which such losses occurred have a bronze colored aleurone since they carry the *C* and *bz* alleles and also have red-staining starch in the underlying endosperm cells due to the uncovering of the *wx* allele. She observed that the aleurone cells in the transitional zones between *C bz* (bronze color) and *I Bz* (colorless) sectors had a deep color similar to that found in *C Bz* cells. She concludes that a substance produced by the *Bz* allele in the colorless (*I Bz*) sectors diffuses into *C bz* areas where, in the presence of the *C* gene, purple or red aleurone color is formed which is similar to that found in cells with both the *C* and *Bz* genes.

The transition zone with purple or red pigment is several cells in width, but the greatest intensity of color is found in a single row of cells near the middle with progressively lesser amounts of pigment in those cells lying to either side. Our observations on material generously furnished by Dr. McClintock indicate that in the transition zone the middle row of aleurone cells with the greatest amount of pigment is *C bz wx* genotypically but *C Bz wx* phenotypically since the endosperm cells precisely underlying this row of cells had red-staining starch.

If the type of endosperm starch can be taken as accurately delimiting the *C bz wx* and *I Bz Wx* sectors, and the evidence is that it can, it follows that recognizable amounts of anthocyanins extend through two to three cells into the *I Bz Wx* sector. It is known that the *C* locus (McClintock, 1949) produces a diffusible substance involved in pigment production and one explanation of the presence of anthocyanin in the adjacent *I Bz Wx* areas is that this diffusible product of the gene *C* in the *C bz wx* cells passes into the *I Bz Wx* sector where it interacts with *Bz* to produce anthocyanin. An alternative possibility is that following the diffusion of *Bz* substance from

the *I Bz* sector into the *C bz* cells and the formation of red or purple pigment, there is a later diffusion of the pigment, or its precursor, back into the *I Bz* cells. In either event it appears that though the *I* allele is able to block *C* action in the *I Bz* sectors it does not inhibit color formation when the *C* gene product or pigment precursor moves in from neighboring *C bz* cells.

Recognizable amounts of anthocyanins also are found in the *C bz wx* sectors a distance of three and more cells from the intensely pigmented middle row of the transitional zone. The progressively lesser pigmentation in cells more distant from the *I Bz* sector presumably reflects the diffusion gradient of the *Bz* substance produced in the *I Bz* sector.

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# ON THE DESIGNATION OF CLOSELY LINKED GENES WITH SIMILAR EFFECTS

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Within the past decade there has been increasing evidence for the widespread occurrence of closely linked genes with similar effects. Thus detailed investigations have established that crossing over occurs between such genes in Drosophila (Oliver, 1940; Oliver and Green, 1944; Lewis, 1941, 1945, 1948; Green and Green, 1949), in the mouse (Dunn and Caspari, 1945), in maize (Laughnan, 1949), in *Aspergillus* (Roper, 1950) and elsewhere. In addition there are instances of alleles whose distinctive patterns of mutation or otherwise unique actions or interactions have been taken as circumstantial evidence for their compound nature, though for many of these cases direct proof of the separability of components is still lacking (see Komai, 1950; Bonner, 1950; Stephens, 1951).

There is a growing tendency in the literature and elsewhere to refer to closely linked, though separable, genes with similar effects as "pseudo-alleles." Thus Lewis (1948) applies this terminology to the bithorax-bithoraxoid and the Stubble-stubbloid loci and, by implication, to the Star and asteroid loci also. The use of the term in these instances may stem from the fact that the closely linked mutant forms were considered allelic prior to investigations which indicated they could be separated by crossing over. This appellation may seem to be particularly appropriate for those cases in Drosophila in which the heterozygotes represented as $+_1\ +_2/m_1\ m_2$ and $+_1\ m_2/m_1\ +_2$ ($m$ designates the mutant form) are not equivalent in action (Lewis, 1945; Green and Green, 1949). Thus, by reason of a position effect, the $+_1\ m_2/m_1\ +_2$ individual has a mutant phenotype and falsely indicates allelism between $m_1$ and $m_2$.

On the other hand Stephens (1948) has defined pseudo-allelism as "a situation where two members of a supposedly allelic series give a complementary hybrid when crossed together. Typically the parental types are intermediate members of the 'series' and the complementary hybrid resembles the top dominant or wild type." On this definition $m_1$ and $m_2$ in the generalized case for Drosophila discussed above are not to be considered pseudo-alleles since the $m_1/m_2$ hybrid typically fails to show complementary action; in a recent publication (Stephens, 1951) cases such as these are not included in a discussion subheaded "pseudo-alleles."

It is interesting to note the occasion for the original use of the term "pseudo-allelism" (Morgan, Bridges and Schultz, 1938). Bridges describes five independently occurring dominant vestigial mutations. The homozygotes of each, and the heterozygotes between them in all possible combinations, were found to be lethal. Crosses to vestigial in each case produced hybrids

with an exaggerated vestigial phenotype. The deficiency nature of these mutations was confirmed in crosses to l(2)C (a lethal factor located 0.1 unit to the right of *vg*) which gave in all cases lethal hybrids. Likewise, four of the five dominant vestigial mutants in heterozygotes with scabrous (a rough-eye character 0.3 units to the left of *vg*) showed the scabrous character. Thus the vestigial mutants give a positive though spurious test for allelism with three closely linked, recessive factors whose phenotypic effects are quite unrelated. In Bridges' words (page 305), "It is considered that any dominant mutant which is lethal when homozygous and which shows pseudo-allelism to a dissimilar, non-allelomorphic (sic) but neighboring mutant is probably a deficiency."

McClintock (1944) describes a somewhat analogous case in maize involving terminal deficiencies of the short arm of chromosome 9. The mutant *wd*, identified cytologically as a deficiency, gives a test for allelism with the recessive mutant yellow-green 2 ($yg_2$) and with *pyd*, a form associated with pale-yellow phenotype and itself established as a deficiency for a terminal piece of chromosome 9 of lesser extent than that involved in *wd*. Here again two separable loci, $yg_2$ and *pyd*, are found to be allelic with a deficiency, *wd*. McClintock states, "According to Bridges these mutants are 'pseudo-allelic.' The term 'pseudo-allelic' presupposes a knowledge of some special alteration which accompanies the expression of allelism."

Thus the original use of the term "pseudo-allelism" was intended to describe an anomalous situation wherein, due to some special chromosomal alteration, genes which are not strictly alleles nevertheless give a test for allelism. In this sense some argument may still be made for applying the term to those cases in Drosophila in which the heterozygote between single mutant forms at closely linked loci, by reason of a position effect, gives a false indication of allelism. It would be presumptuous, however, to designate all closely linked genes with similar effects as pseudo-alleles on this basis since this special kind of position effect has been shown only for Drosophila. Moreover, since the term as originally used describes an "allelic" relation between genes having greatly different phenotypic effects and probably a fortuitous proximity it can hardly be considered distinctive for those closely linked genes whose origin by duplication is suggested by their similarity of action and in some cases by independent cytological observations.

Other names have been suggested for linked genes of this kind, viz. "partially allelomorphic" (Agol, 1930), "twin genes" (Komai, 1950) and "semi-allelic" (Muller, 1949; Komai, 1950). However, none of these takes account of the two attributes which gene systems of the type discussed here have in common, namely, close linkage between members of the group and their similar phenotypic effects. The term "para-allelic" is suggested as satisfying this fundamental requirement. Thus *para* (= beside; alongside of) indicates the anomalous side-by-side arrangement as distinguished from the usual opposite orientation and the word *allelic* implies gene forms with similar phenotypic effects.

It is not intended that this term should designate gene forms as physically discrete entities, but it is implied that there exist closely linked *differences* in chromosome architecture which are separable by crossing over and which determine qualitatively similar deviations from the phenotypic norm. Whether such differences reside in physically isolated entities or are local modifications of a relatively extended determinant (see Goldschmidt, 1950) is open to question, but neither situation appears incompatible with the hypothesis which would account for the origin of para-alleles through intrachromosomal duplication.

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# THE FIRST OCCURRENCE OF THE WHALE SHARK, RHINEODON TYPUS, IN THE WESTERN ATLANTIC— ORMOND, FLORIDA, JANUARY, 1902

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Late in January, 1902, a "sea monster" (a dead whale shark) came ashore on the beach some three miles above Ormond, Florida. Among those who went to see it was Mr. Walter R. Merryman, an advertising man doing business with the Hotel Ormond. Fortunately, when he went to see the "monster," he carried his camera and hence was able to make a photograph of the stranded whale shark. This is the first occurrence of a Rhineodon in Florida waters, and, indeed, in the Western Atlantic, and the photograph is possibly the first ever made of this great fish anywhere.

The managers of the Hotel Ormond had wired a description of the shark (18 feet long) to the Smithsonian Institution, and were asked to send the skin on to Washington at once. That this great fish was in bad condition was to be expected since it had apparently been dead for days. This bad condition can be readily seen by studying the photograph (fig. 1).

How the skin was removed will be detailed in a following paragraph, but it may be stated here that one cannot "husk the hide" off a shark as one can from an ox. In sharks the inner skin tissues are very closely applied to the body wall musculature, and one has to cut the flesh away from the shark's skin. That the skin of this whale shark was removed and that it reached the Smithsonian Institution is known from a note by B. A. Bean in *Science*, 1902, vol. 15, p. 353. Here he correctly states that this is the first record of the occurrence of Rhineodon, not merely in Florida waters, but on the Atlantic coast of America. At this stage in this study a letter was sent to Dr. L. P. Schultz, the present curator of fishes in the United States National Museum, Washington, D. C., asking for information about this skin. Dr. Schultz most opportunely sent a copy of the letter, dated January 27, 1902, of the managers of Hotel Ormond to the Smithsonian about the skin. From this the following detailed data are taken.

This Rhineodon, 18 feet long (it reaches 60 feet), is one of the smallest specimens on record, but it was so heavy and so flattened by beginning decomposition that it could not be moved by manpower—"a pair of heavy draft horses was required to turn the shark over on its back" so that the two skinners could slit the hide down the center of the ventral surface and each man have a half of the skin to work on. This was done, but it took the two skinners (one a professional tanner), with the help of other men to hold the skin free from the body, the whole day to cut the skin free from the body musculature. However, they at last got it free from the carcass, and then they could cut stray bits of flesh from it. Then the skin was rubbed

FIGURE 1. The dead whale shark stranded on the shore three miles above Ormond, Florida, in late January, 1902. Note the flattened body, the general dilapidated condition, and the mutilated tail fin. Photograph by courtesy of W. R. Merryman, 1902.

with a mixture of salt, saltpeter and alum—especially the head parts and about the bases of the fins. At the end of this strenuous day, the men got back to Hotel Ormond with the skin about sunset, January 25, 1902. The next day the skin was rolled up in salt, packed in a cask with more salt, and carried to the railway station to be sent by freight to Washington.

Two photographs were made: one of the fish in side view to show the whole body in lengthwise extent, and the other a foreshortened view of the head to show its great width. These photographs have unfortunately got lost, hence the great value for this paper of Mr. Merryman's photograph (fig. 1 herein), so fortunately preserved.

That the skin reached the Smithsonian is evidenced by the note by B. A. Bean in *Science,* and Dr. Schultz writes that it is there today, kept in a room where skeletons and other dry materials are stored. Evidently it was in such condition when received that it could not be tanned and mounted.

We now return to Mr. Merryman's 50-year old negative portraying our fish. This, together with many other negatives, was packed up and put away and forgotten. Recently, however, when he read Heyerdahl's book, "The Kon-Tiki Expedition," and its account of the encounter of the raft with a whale shark in the South Pacific, it brought to mind his negative of the Ormond specimen of January, 1902. He hunted out the negative, made a print from it, and sent it to Dr. R. C. Murphy in our Museum (for whom he had made photographs of oceanic birds in the past). Together

with a picture of the whale shark came a long letter giving many of the facts synopsized herein. Dr. Murphy brought the photograph and letter to me with the cheerful remark—"You are the chief whalesharker in these parts and here is something that will be of interest to you." So now, after these long years, I am enabled to describe the first Florida whale shark and publish its photograph—January 27 (?), 1902.

The coming ashore of this dead Rhineodon is a rather notable thing. So far as I know, but one other such occurrence has ever been noted. It is recorded that in February, 1889, a 22-foot specimen was stranded on a beach near Madras, India. That these two dead Rhineodons have drifted ashore is, in the light of what is now to follow, truly remarkable.

Whale sharks are not infrequently killed by steamers. I have put on record more than a dozen cases (four in one article) of such occurrences. These great lumbering sharks grow to 60 feet in length and are the largest marine animals, save only the whalebone (blue) whales, which grow to 80 and 100 feet. These giant sharks have no known enemies. A Rhineodon, swimming along the surface, blindly crosses the track of a steamer and there is a collision. The whale shark's back is broken by the bow of the steamer. The forward movement of the steamer is so impeded that it stops to free itself of the great fish. However, not infrequently the Rhineodon does not drop off, whereupon the steamer has to "back water" to free itself of the incumbrance. What becomes of these dead Rhineodons is not known. Because of its two- or three-inch dense, heavy, shagreen-covered skin, the great shark cannot be torn to pieces by voracious smaller sharks. Presumably it sinks and finally is broken up by bacteria. I have had come to me, as noted, numbers of reports of such steamer accidents to whale sharks, but never one of the coming ashore of such a broken-backed Rhineodon. Furthermore, no such account has been found in the literature.

FIGURE 2. A freshly killed whale shark on the beach at Acapulco, Pacific coast of Mexico. The rollers have humped the fish too much, but in shape, fins and markings, it is a splendid specimen. Photograph by courtesy of Ralph L. Smith, 1935.

But let us return to the Ormond stranded specimen and to its fate. Mr. Merryman's photograph (fig. 1) is surely that of a whale shark. Note the distinguishing characters; the greath breadth anteriorly, the wide terminal mouth, the longitudinal ridges on the side, the white spots, the prominent dorsal fin and finally the tall caudal fin. It is a whale shark, surely, but a very dilapidated one—dead no one knows how many days. The tip of the upper lobe of the tail fin has been cut off and the hinder edge of this upper lobe has suffered some mutilation at the hands and knives of curio seekers. But for all these things, it is a Rhineodon—the first to be recorded from the Florida waters and from the Western Atlantic.

This Rhineodon should be contrasted with that shown in fig. 2. This is made from a photograph of a recently killed and still fresh whale shark dragged up on the beach at Acapulco on the Pacific coast of Mexico in 1935. This Acapulco shark was so big and heavy that, to get it on the beach, a temporary track with movable rollers had to be built out in the water. Then the Rhineodon was floated on to the rollers and, as the tide fell, it was dragged up on the beach. The rollers have unfortunately humped the body up too much, but allowance can readily be made for this. Withal, it is plainly a fresh and unmutilated specimen of a splendid whale shark. Let the reader contrast it with the dilapidated specimen portrayed in fig. 1.

Now but a few words are needed as to the subsequent history of the remains of the Ormond Rhineodon. So long as the thick, armor-like skin was intact, the vultures ("turkey buzzards") could do nothing to the dead fish. But Mr. Merryman notes that, when the skin was finally removed, the birds descended on the remains in flocks and soon there was nothing left but the cartilaginous skeleton.

This, then, thanks to Mr. Merryman and Dr. Schultz, is the history of the first Florida whale shark—practically fifty years ago. The published accounts of other and later specimens from Florida, the Gulf of Mexico, and from West Indian waters would make a small volume.

# LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

## UNORDERED TETRADS

The following problem was presented to me by Dr. C. C. Lindegren and its solution may be of interest to other micro-geneticists: What is the probability of getting two identical tetrads when n independent factors are involved and spores are collected in unordered tetrads?

It is convenient, for the present purpose, to divide the various kinds of possible tetrads into two genera, those in which all four spores are of different genotype, and those in which there are two pairs of identical spores. In Table 1 various useful quantities are shown in respect to these two genera.

TABLE 1

| | 2-type genus | 4-type genus | both genera |
|---|---|---|---|
| (1) No. of kinds of tetrad pooled in each unordered tetrad of this genus | $4!/2! \times 2! = 6$ | $4! = 24$ | |
| (2) No. of different kinds of unordered tetrads in this genus | (3) $2^{n-1}$ | $\frac{6^{n-1} - 2^{n-1}}{4}$ $[(4) - (3)]$ | $(4)^1 \frac{6^{n-1} + 3 \times 2^{n-1}}{4}$ |
| Total kinds of tetrad i.e. (1) × (2) | $6(2^{n-1})$ | $6(6^{n-1} - 2^{n-1})$ | $6^n$ |

The total number of different kinds of tetrad is $6^n$ but in unordered tetrads these cannot all be distinguished. The first row in the table shows how many of the total number of different kinds of tetrad are pooled in any particular unordered tetrad.

Assuming that the $6^n$ different tetrads occur with equal frequency, the probability that any unordered tetrad in the 2-type genus will be of a particular kind is $6/6^n$. The probability of getting two unordered tetrads in the 2-type genus of the same particular kind is $(6/6^n)^2$, but since there are $2^{n-1}$ kinds of unordered tetrad in this genus the probability of any two unordered tetrads being identical is $(6/6^n)^2 \times 2^{n-1}$. By a similar argument the probability of getting two identical tetrads in the 4-type genus is $(24/6^n)^2 \times (6^{n-1} - 2^{n-1})/4$. The probability that any two unordered tetrads will be identical is the sum of these probabilities: $(4 \times 6^{n-2} - 2^{n-2})/6^{2n-3}$.

The corresponding probabilities of getting three or more identical tetrads can be arrived at by a similar method. In general the probability that any i unordered tetrads will be identical is: $(6/6^n)^i \times 2^{n-1} + (24/6^n)^i (6^{n-1} - 2^{n-1})/4$.

In practice these will be minimal probabilities for where segregation is not completely independent there will be more heterogeneity and this will better the chance of getting identical tetrads.

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## THE HEPATIC VENOUS THROTTLE MUSCULATURE, A MECHANISM HARMFUL TO THE INDIVIDUAL BUT PERHAPS BENEFICIAL TO THE SPECIES

In 1915 Mautner and Pick[1] found that anaphylactic shock in dogs was a result of blood retention in the liver. They hypothesized that a sphincter mechanism might exist in the walls of the hepatic veins. Their hypothesis was subsequently verified by several investigators[2,3,4] Arey[4] and Stark[5,6,7] demonstrated this musculature in various other mammalian species, and Elias and Bengelsdorf[8] added a fish to the list, namely, the carp. The musculature consists of strong bands arranged around the veins in spiral and ring form. They accompany the major part of the system of the hepatic veins from the central veins to large, collecting veins.

The remarkable thing about these muscles is that they exist only in a small number of isolated species, (Arey, 1941; Elias and Bengelsdorf, 1951) while even closely related species do not have them. Obviously, since these throttle muscles are absent in the majority of species, they cannot be necessary for life. It is admitted that they can perform a useful function regulating the blood flow through the entire liver and even through restricted regions or individual lobules, and that they can bring about blood storage. Yet, the fact remains that most vertebrates live without these muscles. In other vertebrates, the storage phase is brought about through the contraction of the "outlet sphincters," i.e. contractile parts in the walls of individual sinusoids near their entrance into a central vein, demonstrated by Knisely, Bloch, and Warner in 1948[9].

In the dog, however, the hepatic venous throttle musculature can cause death during anaphylactic shock. In other words, its specific function which distinguishes it from that of the outlet sphincters of the sinusoids, is dangerous to the life of the individual. Yet this mechanism is not dangerous enough to have wiped out an entire species.

Two interpretations of the significance of the throttle veins are possible, from the standpoint of evolution: (1) The mechanism has no significance, it is neither useful, nor harmful enough to endanger the life of the species; (2) The mechanism causes the death of highly allergic individuals and, thus, improves the general health of the species.

A similar mechanism may be found in the bronchial musculature of all mammals. Asthma consists in a contraction of the bronchial muscles as a response to an allergy. While in our civilized society an asthmatic patient can prolong his life by rest and treatment, under uncivilized conditions asthma is much more dangerous, if only because the sufferer cannot run away from wild beasts. Thus, the bronchial musculature may have eliminated, in the past, allergic individuals from our own species before reproductive age and, thus, it may have helped to keep the species as a whole more healthy.

This thought is, herewith, submitted as a mere hypothesis.

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## ADAPTEDNESS OF INDIVIDUALS AND OF POPULATIONS

The interesting note of Dr. Hans Elias raises issues of so general a significance that further discussion seems justified. The throttle musculature of the hepatic veins exists only in some species in various groups of mammals, while it is absent in most other species. It is argued on this basis that the presence of this musculature is not essential for life. On the other hand, the throttle musculature may be supposed to be useful to the species, rather than to individuals, since it may cause death of allergic individuals and thus keep up the adaptedness of a species as a whole.

Now, the fact that a trait occurs only in some species does not prove that it has no adaptive significance in individuals of the species in which it is normally present. For example, the ability to withstand cold may be unimportant in the inhabitants of the tropics, but essential in temperate and cold regions. Concealing and warning colorations and resemblances occur by no means in all animals, but they may be very useful where they do occur. To prove that the hepatic throttle musculature is of no adaptive significance, one should compare, in a variety of environments, individuals which have it with individuals which have lost it through mutation or other means. If such a comparison is impossible, then adaptive value of a trait may sometimes be inferred by comparison of the physiologies and ecologies of species in which the trait is present with those in which it is absent.

Natural selection favors the establishment of genetic variants which are immediately useful, regardless of their eventual value or harm to the species. Suppose that a population is a mixture of healthy individuals and of individuals genetically predisposed towards allergies. The reproductive potential of allergic individuals being impaired, natural selection will tend to eliminate the allergic genotypes. Another genetic variant, which causes the development of the hepatic throttle musculature, makes the carriers of the allergies inviable. The selection pressure will then increase not only against the allergy carriers but against the possessors of the throttle musculature as well. In other words, the hepatic throttle musculature cannot develop and become established within populations which contain allergic individuals. This is not a probable mechanism for keeping up the adaptedness of a species.

And yet, it is true that traits which are useful to a population may be established by natural selection, even though they may be deleterious to some individuals. In sexually reproducing organisms, individuals as well as Mendelian populations are the units with which natural selection operates. This is shown especially clearly by the phenomenon of balanced polymorphism, where selection maintains poorly adapted homozygotes in populations, provided that the heterozygotes possess a superior fitness. A species is only the most inclusive Mendelian population. A mechanism of the sort postulated by Dr. Elias could, then, operate as follows. Suppose that we have a series of competing Mendelian populations (races or

species), with varying frequencies of the genes for allergies and for the hepatic throttle muscles. The lowest adaptive values will occur in populations in which both of these genes are frequent; populations with allergies but without the hepatic muscles will be somewhat better off; and populations in which the muscle trait is frequent but the allergy trait is rare, and those in which neither trait is frequent will be the most fit. This presupposes, of course, that the presence of the throttle musculature is not per se useful; if it is, the trait will be selected for regardless of its interaction with the allergies.

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## A PRELIMINARY NOTE ON THE BIOLOGY AND CHROMOSOME CYCLE OF *APHIOCHAETA XANTHINA* SP

*Aphiochaeta xanthina* Speiser (Dipt. Brachycera, Fam. Phoridae) has been described by Speiser in 1907[1]. According to this author, the specimens, collected in Cameroon, belong to the genus Aphiochaeta with three closely related species: *Braunsi, lutea* and *atlantica.*

An undetermined species of the same genus has been submitted to cytological and genetical investigation by Tokunaga (1949, 1950)[2] who accounts for a haploid number of 3 chromosomes, and describes several spontaneous and induced mutants. The chromosome counts were made on both ganglion and gonad cells.

The present material, kept in culture since 1946, was originally collected by Dr. G. Bertani at Naples, who began some investigations on the chromosomes (unpublished). The systematic identification (made by Dr. E. Séguy,* to whom we are glad to express here our thanks) permits us to consider the material as *Aphiochaeta xanthina.* Data on the biology of this species, as well as on the chromosome cycle are presented here, as an introduction to further cytological and genetical investigations.

(A) *Development. A. xanthina* lays numerous eggs on standard maize meal food for Drosophila, and development of larvae, as well as pupation and further metamorphosis, take place normally. This food is by no means the best (the data agree with those obtained and privately communicated, by Dr. G. Bertani), as is proved by the scarcity of offspring. Since the wild Aphiochaeta feeds on meat of dead animals, a food has been composed by adding amino acid preparations (as "Bioaminoidi," commonly used as a pharmaceutical product) or liver powder to the Drosophila culture medium, in varying proportions. The best proved to be 6 percent of liver powder, with maize meal, agar and glucose in water solution, in the usual percentages as for preparing Drosophila cultures. A higher proportion of liver powder (12 per cent) inhibits the development.

The developmental period comprises 5–6 days for embryonic development and larval life, and 11–13 days for pupation and further metamorphosis, i.e., about 18 days. These data represent the average from 41 cultures. The parents remained in the culture bottle for one day. The emerging of imagines was scattered over a period of nearly 6 days. Twenty single pair cultures (with virgin female), gave a total of 643 individuals ($32.1 \pm 4.4$ per culture), of which 361 were males and 282 females.

The per cent of males, $56.14 \pm 1.97$, indicates with high probability $\left(p = .003, \frac{\text{dev.}}{\text{S.D.}} = 3.11\right)$, that the adults counted deviated significantly from the expected ratio 1 : 1. This observation is, however, not sufficient to suppose a peculiar mode of sex determination, because a different duration

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of life or a different degree of viability of the sexes might explain the male surplus.

(B) *Chromosome cycle.* The chromosome cycle has been studied mainly in the mitosis of the ganglion cells and in the male meiosis, using aceto-carmine and orcein squashes. The somatic metaphase shows 10 acrocentric, rod-shaped chromosomes of uniform, even if not equal, size, and is not distinguishable in both sexes. They tend to pair at one end, so that 5 V-shaped bodies result. The anaphase shows clearly, on the other hand, that no mediocentric chromosomes are present. During the resting stage, one big nucleolus appears, but no chromocenter. The male meiosis shows moderately clear leptotene and zygotene, but a typical pachytene, where pairing is complete, and no heteropycnosis can be found. Diplotene with chiasmata is also clear. A strong contraction takes place during diakinesis, and a 1st metaphase is formed with 5 gemini of uniform size and terminalized chiasmata. The second division follows regularly. The salivary nuclei show a peculiar structural condition.** Polytenic structure appears only if the food is rich in amino acid and fats (liver powder). On the contrary, with a poor diet, the salivary nuclei appear as polyploids with filamentous chromatin, but fail to show any polytenic longitudinal association. The salivaries with the typical banded structure break spontaneously, when crushed for squashing, and thus are not suitable for cytological investigation. They do not show any chromocenter, except a small corpuscle closely linked with the nucleolus. As a preliminary conclusion, it can be said that *Aphiochaeta xanthina* Sp. can supply good material for both genetical and cytological investigations.

When compared with male meiosis of other Diptera, the spermatogenesis of this species seems exceptionally regular. Normal disjunction and crossing-over in the male can be expected. The detection of chromosome mutation is not likely to be shown by salivary chromosomes, but pachytene stage seems to be more suitable for that purpose.

The discrepancy in chromosome number between Tokunaga's material and our own cannot be discussed, because we do not know whether the investigations have been carried out on the same species.

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**Dr. G. Bertani's unpublished data agree with our observations.

## PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

EDITORIAL OFFICE
The American Naturalist
635 W. 247 St.
New York 71, N. Y.

Bates, Marston, 1952. Where winter never comes. 310 p. $3.50. Charles Scribner's Sons, New York.

Baumgardt, Carola, 1951. Johannes Kepler: life and letters. 209 pp., $3.75. Philosophical Library, New York.

Extracts from Kepler's letters are skillfully joined by a narrative text to make a plausible sketch of his scientific personality against the intellectual and political background of the times. This unpretentious book seems a valuable contribution to the biographical and historical approach to the understanding of science.

M.B.

Blum, Harold F., 1951. Time's arrow and evolution. 222 p., 20 figs., 4 plates. Princeton University Press, Princeton, N. J.

Sir Arthur Eddington, in calling the second law of thermodynamics "time's arrow," implied that this law points the direction of all real events in time; and Blum here has carried out thought-provoking explorations of the relationships between the physical and chemical concepts arising out of this second law, and the processes of organic evolution. He is thus concerned with energetics and kinetics, with the history of the earth, with the fitness of the environment, with the nature of living systems, and with the problems of the origin of life.

M.B.

Bullough, W. S., 1951. Vertebrate Sexual Cycles. 117 pp., 12 text figures. $1.50. J. Wiley and Sons, New York.

This is a valuable addition to Methuen's Monographs on Biological Subjects, presenting in brief compass and at reasonable price the essential facts concerning the oestrous cycle in female mammals and seasonal reproductive cycles in vertebrates generally.

L.C.D.

Darwin, Charles. On the origin of species by means of natural selection. A reprint of the first edition, with a foreword by C. D. Darlington. 426 pp., 1951. $3.75. Philosophical Library, New York.

The first edition of Darwin's classic is practically unknown to modern readers, since the numerous reprintings and translations have been made from later editions, in which the manifold èmendations and compromises have, perhaps, impaired the clear and direct argumentation of the original. A foreword by C. D. Darlington is lively and aggressive.

T.D.

Dobzhansky, Th., 1951. Genetics and the origin of species. 3rd ed., revised, *X*. 364 pp. 23 figs. Columbia University Press, New York.

The significant developments in the study of evolution in the last decade were twofold. Firstly, genetic principles have invaded systematics, ecology, paleontology and other biological disciplines impinging on the subject. Secondly, population genetics, following the antecedent theoretical and descriptive approaches, flowered on the experimental level. In both of these developments the leading influence belongs to the earlier editions of this book. The current revision of Dobzhansky's classic may then be viewed as an authoritative though impersonal survey of what the preceding versions have wrought in genetics and in the broader purlieus of biology as a whole. Thus not only does the book give an up-to-date picture of our understanding of evolutionary processes from the genetic point of view but, by comparison with the second edition, it affords an estimate of the effects that the most influential work of the century in this field has had on biological thought.

The greatest similarities between the new and the old editions are in the overall viewpoint, in comprehensiveness, incisiveness, lucidity and felicity of style. The greatest differences are in the vast amount of new experimental data discussed (probably by far the better half of the previous citations have been replaced by more recent material), in organization and elimination of sections on matters now generally accepted, and, as pointed out by Dobzhansky himself, in a greater emphasis on declarative rather than dialectic treatment. Irrespective of exposure to the earlier editions, acquaintance with this revision is a categorical necessity for every biologist.

M.L.

Fuller, Harry J., 1951. The plant world (Revised edition). 769 p., ill. $4.75. Henry Holt and Company, New York.

Gilbert-Carter, H., 1951. Glossary of the British flora. 79 p., $1.75. Cambridge Univ. Press, New York.

Greeley, William B., 1951. Forests and men. 255 p. $3.00. Doubleday and Company, Inc., New York.

Hass, Hans, 1951. Diving to adventure; the daredevil story of hunters under the sea. 280 p., 58 photographs. $3.75. Doubleday and Co., New York.

An account of fishing with goggles or helmet, harpoon and camera, in the Mediterranean and off Curacao in the West Indies. The book has no scientific pretensions, but the fish stories are plausible enough and the photographs are splendid.

M.B.

Kemp, Tage, 1951. Genetics and Disease. 330 pp. with 99 text figures. Ejnar Munksgaard, Copenhagen.

This is a very condensed account of a large subject, including as it does a brief introduction to the principles of heredity (105 pp.), medical genetics (27 pp.), normal heredity in man (56 pp.), hereditary diseases (124 pp.). Most subjects are treated in outline form rather than exhaustively, but a special effort was made to treat blood groups more fully, including the newer factors, Kell, Duffy, Lewis, Lutheran, and the linkage between the last two. The book is probably devised primarily for the instruction of medical and premedical students and is complete enough to serve as an introductory text.

L.C.D.

Metcalf, C. L. and W. P. Flint, 1951. Destructive and useful insects; their habits and control. Third edition, revised by R. L. Metcalf. xiv, 1071 pp., 584 figs. $10.00. New York: McGraw-Hill Book Co.

This elaborate textbook, or reference book, for economic entomology in North America, tells how to identify pests, catalogues the variety of damage that they cause, and provides a manual of strategy and tactics for "the war against insects." Some attempt is made to balance the usefulness of the honey bee, the silkworm and the pollinating bumble bee against the black record of the boll weevil and the corn borer; but the book conveys no feeling for insects as part of a total ecological situation. In this the book reflects accurately the preoccupations of most American economic entomologists.

M.B.

Mühldorf, Anton, 1951. Die Zellteilung als Plasmateilung. 194 p., ill. $4.70. Springer-Verlag, Vienna, Austria.

Raman, C. V., 1951. The New Physics. 144 p. $3.75. Philosophical Library, New York.

Rodgers, Andrew Denny, III, 1951. Bernhard Eduard Fernow. 623 p. $7.50. Princeton University Press, Princeton, N. J.

Saunders, Aretas A., 1951. A Guide to bird songs. 307 p. $3.00. Doubleday and Company, Inc., New York.

## ADVICE TO AUTHORS

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